GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
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CORRIGENDA

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Page 92, line 16, for "1901" read "1921."

Table 4, facing page 104, top of "parents" column for "Stock14 ♀ ♀ ×
stock 3 ♂ ♂"
read "stock 3 ♀ ♀ ×
stock 14 ♂ ♂"

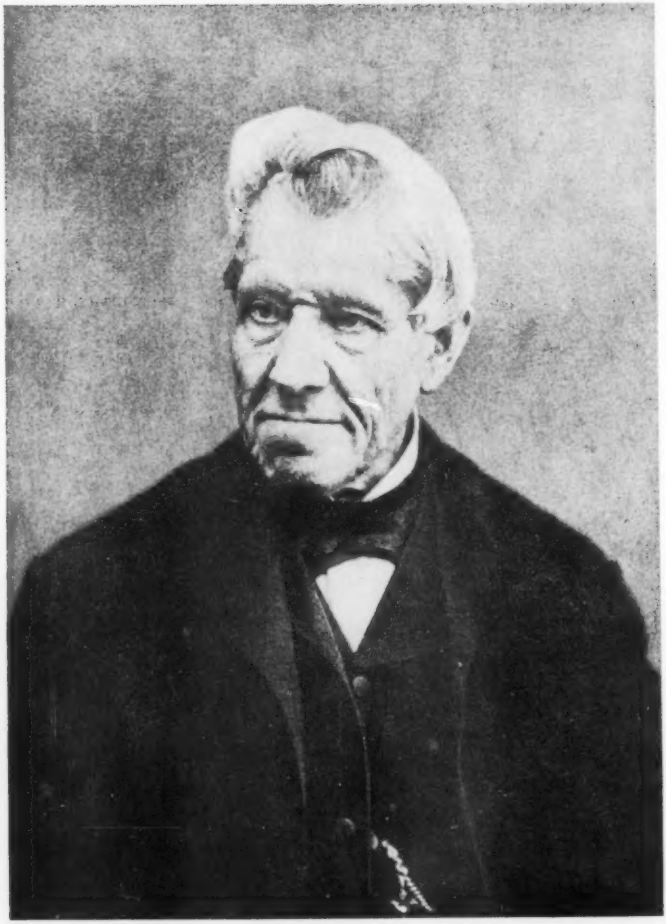
Page 275, second line from bottom, for "the" read "some" and for "are' read "were"

Page 574, line 6, for "d^3" read "$\overline{d}^3$."

Page 574, line 8, for "$\overline{d}^3$" read "$\overline{d}^3$."

Page 574, formula just before table 7, for "$\overline{d}^3$" read "$\overline{d}^3$."

Page 574, third and line fourth of the first column of table 7, for "$\overline{d}^3$" read "$\overline{d}^3$."



H. Jordan

ALEXIS JORDAN

(Frontispiece)

ALEXIS JORDAN was born in Lyon, France, October 29, 1814, of an old and distinguished Lyonnaise family, and he died suddenly in the same city in his 83rd year, February 7, 1897. At an early age he became interested in the local flora about Lyon, and later during a period of thirty years following 1836, he made many excursions to the various parts of south-eastern France, especially devoting many of these collecting trips to mountainous regions surrounding the valley of the Rhone, and in Corsica.

While his interest was wholly taxonomic, his work is of special interest to geneticists, because he was the first to carry the genetical concept of species to a strictly logical conclusion. In 1869 he wrote to a botanical colleague: "*Prouver qu'une forme végétale est héréditaire et permanente, c'est prouver qu'elle est une espèce.*" In order to test the constancy of the characters that his keen powers of observation detected in nature, he established in 1844 an experimental garden at Cité-Villeurbanne where he grew progenies from the wild forms he had distinguished, and compared these progenies with one another, often through a long succession of generations. His descriptions were written for the most part from these experimental cultures; the first of his 45 published papers appeared in 1846 and the last was published posthumously in 1903. In later years (after 1877) he ceased to make collecting trips himself but continued testing in his experimental garden the lots of seeds received from many collectors.

His published papers,—some of these prepared in collaboration with one or other of his two gifted young assistants, JULES FOURREAU, and HILARION BOREL,—contain the names and descriptions of nearly 1700 new species. Many other elementary species,—"Jordanian species" or "Jordanons," as they have often been called,—were recognized, but have never been published. The relation of these "*petites espèces*" to the conglomeration of hereditarily distinct forms which make up the "Linnean species," or "Linneons" may be best indicated by citing a few cases. In *Saxifraga aizoon* 22 new forms were found to be hereditarily distinct and constant and were published as new species; in *Artemisia campestris* 25; *Scabiosa succisa* 25; *Betonica officinalis* 27; *Narcissus tazetta* 32; *Sempervivum tectorum* 35; *Erophila vulgaris* (*Draba verna*) 200. In the last-mentioned Linneon only 55 of the 200 recognized forms were published, twenty of them with beautifully executed colored plates. In cultivated

plants he also found similar examples of true-breeding components. For example, in a few years time he was able to recognize more than one hundred distinct biotypes of wheat.

Needless to say, these results were not accepted sympathetically by JORDAN'S taxonomic contemporaries who dubbed him a frenzied species-maker, counter of hairs, and so forth, overlooking the long years of careful experimental work on which JORDAN'S new forms were based.

JORDAN mingled little with society, preferring to work in solitude and it is not unlikely that the bitterness of the criticism and the ridicule of his contemporaries had much to do with the fact that he acquired the reputation of being a misanthrope.

JORDAN was a devout churchman of the Roman Catholic faith and as his work was already well advanced before the publication of DARWIN'S "Origin of Species," it is not surprising that he maintained consistently throughout life that every true-breeding form of plant or animal represents a distinct design on the part of the Divine Mind.

JORDAN'S experimental garden, unique of its kind, was abandoned in 1902, the year of the death of HILARION BOREL, and his botanical library which was perhaps the largest and most complete which had been accumulated by any individual botanist, was sold in 1903 for 63000 francs. He accumulated one of the most extensive private herbaria of Europe, which contained not only the very numerous collections of himself and his assistants, but also about 200 sets of exsiccati purchased from other collectors. Unfortunately this collection does not contain all of the elementary species published by JORDAN, though it does contain many which were named by him but never published. This herbarium was given by his executor to the CATHOLIC UNIVERSITY OF LYON, where it is available to other botanists who desire to consult it.

ALEXIS JORDAN became a member of the ACADÉMIE DES SCIENCES, BELLES-LETTRES ET ARTS DE LYON in 1850, of the SOCIÉTÉ D'AGRICULTURE and the SOCIÉTÉ LINNÉENNE DE LYON about the same time; of the SOCIÉTÉ BONTANIQUE DE FRANCE in 1854, the date of its founding; the ASSOCIATION FRANÇAISE POUR L'AVANCEMENT DES SCIENCES from its founding in 1871; of the SOCIÉTÉ DES SCIENCES NATURELLES DE CHERBOURG; the SOCIÉTÉ ROYALE DE BOTANIQUE DE BELGIQUE; the SOCIÉTÉ POLLICHIA OF BAVARIA; SOCIÉTÉ BOTANIQUE DE RATISBONNE and the SOCIÉTÉ IMPÉRIALE DES NATURALISTES OF MOSCOW.

The portrait here reproduced was published in the Annales de la Société bontanique de Lyon, volume 22, 1897. We are indebted to J. MARION SHULL of the U. S. DEPARTMENT OF AGRICULTURE for locating this portrait and providing a photographic copy of it. Doctor HAJIME

UDA, while spending a portion of last year in Lyon, secured and submitted valuable information. Doctor CLAUDIUS ROUX, Secrétaire Générale de L'ACADÉMIE DES SCIENCES, BELLES-LETTRES ET ARTS de Lyon, submitted an alternative portrait, and Monsieur AUG. CHEVALIER, of the LABORATOIRE D'AGRONOMIE COLONIALE, Paris, provided a photographic copy of a letter from which Monsieur JORDAN's signature has been engraved. To all these, the Editorial Board expresses its cordial appreciation.

GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS
II. THE POLYCLADOUS CHARACTER

CHARLES E. ALLEN
University of Wisconsin, Madison, Wisconsin

Received June 30, 1924

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INTRODUCTION

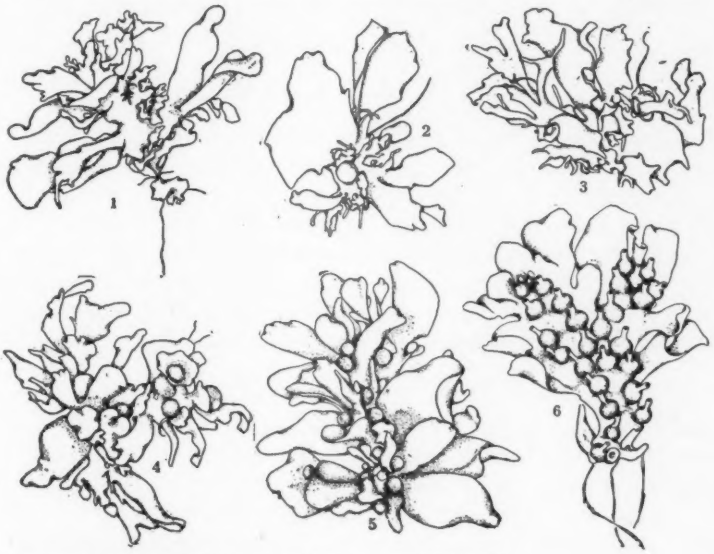
The races to which the term "polycladous" is applied are derived from two male clones (R19δ1aA and R19δ1aB). The two atypical plants with which these clones began were obtained from a pot to which had been transplanted for purposes of fertilization a male clone of known origin that previously had appeared quite typical, and a group of plants, apparently all female, derived from a mixed culture. The two atypical plants when first seen were growing close together and were probably of common origin. They may represent the result of a mutation within the typical male clone, but more probably they (or their common ancestor) were transplanted from the mixed culture with the female plants, being at first overlooked because of their small size.

As will appear, polycladous plants differ so greatly from those of typical races that it might well be questioned whether they are to be thought of as within the limits of the species *Sphaerocarpos Donnellii* Aust. They are provisionally so considered, however, because the polycladous males cross freely with females certainly of this species; whereas numerous attempts to intercross this species with the very similar *S. texanus* Aust. have uniformly failed. Polycladous plants of both sexes have been found by Mr. A. M. WOLFSON to possess chromosome complements similar to those of typical plants of *S. Donnellii* and *S. texanus* of corresponding sex.

The characteristics of the polycladous races will be more fully described in a forthcoming paper by Mr. WOLFSON. It will suffice here, therefore, to note the more conspicuous differences between these and typical races. Polycladous males are distinguished by crowded and very variously formed lateral lobes, sometimes bearing tooth-like or ciliate marginal outgrowths; by irregular, often profuse branching, which suggested the designation "polycladous"; by the frequent appearance of dorsal lobes (rare in typical clones) as well as of dorsal cilia—single rows of cells, variable in length; by the scarcity of antheridia, which are often quite absent in a large portion, or at times throughout the whole, of a culture; and by the usual great reduction of the involucres, the antheridia, in consequence, being commonly exposed. Involucres at times seem to be entirely absent; at other times they are saucer-shaped, or laterally expanded and then leaf-like, lacerate, or ciliate. The dorsal cilia previously referred to probably often represent reduced and dissected involucres. Occasionally an involucre encloses and extends beyond its antheridium, more or less closely approximating, and rarely attaining, the form of a typical involucre. In spite of considerable intraclonal variation with respect to each of these characteristic peculiarities, no polycladous clone has been observed to produce even an approximately typical branch. In their uniformly atypical character, polycladous clones—including those of the f_1 and later generations—differ from the tufted clones described in a previous paper (ALLEN 1924). Polycladous male cultures often grow more vigorously than typical male cultures; when healthy, they are bright green, whereas typical males in greenhouse cultures are commonly yellowish green.

Polycladous males, all from clone R19δ1aB, are shown in figures 1 to 5, in contrast with a typical male plant (figure 6) drawn to the same scale. The polycladous plants shown in these figures illustrate in varying degrees the irregularity of branching, the varied forms of lateral lobes, and the dorsal outgrowths (lobes and cilia—the latter shown most abundantly in figure 2). Figure 5 is of a plant bearing (for a polycladous race) comparatively numerous antheridia, all naked, although beside two or three are outgrowths that probably represent involucres. Borne on the plant of figure 4 are two exposed antheridia, one of which (at the lower right of the figure) is subtended by a lobe and a cilium, which together may represent the involucre; and just above is another antheridium, enclosed within an involucre resembling one of typical form except that its neck flares broadly. Figure 2 is of a plant bearing one naked antheridium subtended (above) by a structure of very irregular form. The plants shown in figures 1 and 3 are sterile.

Matings of these polycladous males with typical or tufted females are regularly fertile. It is true that the sporophytes obtained from such a mating (in proportion to the size of the female culture used) were commonly fewer than those from matings of typical × typical, typical × tufted, or tufted × tufted. This discrepancy, however, is probably to be accounted for by the much smaller proportion of antheridia borne by polycladous



FIGURES 1 to 5.—Plants of a polycladous ♂ clone (R19δ1aB).
FIGURE 6.—A typical ♂ plant of clone 19.27. × 10.

than by typical or tufted males. The present paper presents the results of matings of polycladous males, two with typical females and two with tufted females, the males in each case representing one of the original polycladous clones. These matings are numbered consecutively with those described in the first paper of this series (ALLEN 1924). A brief report of some of the work here discussed has already been published (ALLEN 1923). The slight differences between the tables included in that report and tables 1 and 2 of the present paper are due to corrections resulting from further observation, and to the omission from the tabulation of a few clones whose determination seemed not altogether satisfactory.

As in the work previously reported, I have had the assistance at different times of Doctor H. W. RICKETT, Doctor A. M. SHOWALTER, and Mr. J. A.

LOUNSBURY. The drawings accompanying this paper were made by Miss BETTY THORKELSON and Miss IRMA HAAK. The work has been carried on with the aid of grants from the research fund of the UNIVERSITY OF WISCONSIN.

The methods used have been described in earlier papers (ALLEN 1919, 1924).

EXPERIMENTAL RESULTS

Typical ♀ × *polycladous* ♂

Mating 12. 19.24 × R19δ1aB

Fertilized January 24, 1921. ♀ plants with sporophytes dried April 12. Spore tetrads sown October 25. Sporeling transplanted to separate pot January 12, 1922.

The female parent is the clone described as a typical female in the previous paper of this series and used in mating 1.

Spore tetrads from one capsule resulting from the present mating were sown broadcast. Only one sporeling was obtained. The clone to which it gave rise was a typical male.

Mating 13. 19.6 × R19δ1aA

Fertilized January 17, 1920. Tetrads (from capsules A to F) sown March 27, 28. Sporelings transplanted June 26, 1920, to February 1, 1921. Remainder of culture dried March 28. Tetrads (from capsules G and H) sown November 1. Sporelings transplanted February 3 to April 1, 1921.

The typical clone here used as the female parent was derived from a spore from the same parentage, though from another tetrad, as that which gave rise to clone 19.24.

The results of the germination of spores derived from this mating are shown in table 1. Tetrads from capsules A to C were sown individually. Those from capsules D to H were sown broadcast, the tetrads from each capsule in a separate pot.

Classes 1 (typical) and 2 (possibly atypical), as listed in table 1, correspond to those derived in the f_1 generation from a mating of typical × typical (ALLEN 1924). It is not yet certain whether the "possibly atypical" and the "typical" clones are genotypically different; but since both classes result from matings in which no polycladous ancestors are involved, and both show no trace of polycladous characters, they may be grouped together as non-polycladous, in contrast to the polycladous offspring (class 6, table 1). Essentially, then, the present mating gave

TABLE 1

(p_1) 19.6 (♀ typical) × R19&1aA (♂ polycladous).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	TOTAL NON-POLY-CLADOUS	CLASS 6 POLYCLADOUS	TOTAL
Capsule A (F_1)					
f_1 ♀'s	1	0	1	0	1
f_1 ♂'s	0	0	0	1	1
Total	1	0	1	1	2
Capsule B (F_1)					
f_1 ♀'s	1	0	1	0	1
f_1 ♂'s	0	0	0	0	0
Total	1	0	1	0	1
Capsule C (F_1)					
f_1 ♀'s	1	0	1	0	1
f_1 ♂'s	0	0	0	0	0
Total	1	0	1	0	1
Capsule D (F_1)					
f_1 ♀'s	9	1	10	4	14
f_1 ♂'s	14	0	14	7	21
Total	23	1	24	11	35
Capsule E (F_1)					
f_1 ♀'s	3	1	4	1	5
f_1 ♂'s	3	0	3	1	4
Total	6	1	7	2	9
Capsule F (F_1)					
f_1 ♀'s	2	1	3	2	5
f_1 ♂'s	2	1	3	2	5
Total	4	2	6	4	10
Capsule G (F_1)					
f_1 ♀'s	10	6	16	17	33
f_1 ♂'s	8	0	8	6	14
(Sex ?)				2	2
Total	18	6	24	25	49

TABLE 1 (continued)

(p_1) *19.6* (♀ *typical*) × *R19δ1aA* (♂ *polycladous*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	TOTAL NON-POLY-CLADOUS	CLASS 6 POLYCLADOUS	TOTAL
Capsule H (F_1)					
f_1 ♀'s	7	3	10	4	14
f_1 ♂'s	0	0	0	4	4
(Sex ?)				1	1
Total	7	3	10	9	19
Total f_1 ♀'s	34	12	46	28	74
Total f_1 ♂'s	27	1	28	21	49
(Sex ?)				3	3
Grand total	61	13	74	52	126

Sex and character not determined: capsule C, 1; capsule D, 3; capsule F, 3; capsule G, 4; total, 11.

four classes of f_1 progeny: non-polycladous females, polycladous females, non-polycladous males, and polycladous males. As the table shows, there is a deficiency of polycladous as compared with non-polycladous clones in each sex, and in the total including three polycladous clones whose sex was not determined. The possible significance of this difference will be discussed after the results of other matings have been described. The discrepancy between the number of females and that of males is in accord with previous results, and is doubtless due to the lesser viability of the males and their consequent death in larger numbers both before and after transplantation of the sporelings.

Four polycladous male clones of this family differ phenotypically from the p_1 male clone and from the other f_1 male clones classed as polycladous, in the somewhat more frequent—though still comparatively rare—appearance of involucres more or less closely approaching the typical form. Minor differences of similar nature appear between some of the other polycladous clones. Otherwise, all the f_1 polycladous males seem to be substantially alike and similar to the p_1 male. Such differences as have been observed between them were no greater than those between the appearances, at different times, of a single clone. In other words, so far as the study has yet gone, there is no indication that the several characters distinguishing a polycladous race are genetically separable.

Among the offspring of this and similar matings appeared for the first time the class of polycladous females. Something of their appearance is shown in figures 7, 9, 10 and 11, as compared with that of a typical female drawn to the same scale (figure 8). Polycladous females differ from poly-

FIGURE 7.—A polycladous ♀ plant of clone 21.125.
FIGURE 8.—A typical ♀ plant of clone 21.68.
FIGURES 9, 10.—Polycladous ♀ plants of clone 21.56.
FIGURE 11.—A polycladous ♀ plant of clone 21.140. × 7.

cladous males in size of corresponding parts and in rate of growth in about the same proportions as do typical females from typical males. In vegetative characters other than size—including form and frequency of lateral lobes, irregularity of branching, and the presence of dorsal lobes and cilia—polycladous females resemble polycladous males. Whereas

polycladous male plants bear comparatively few antheridia and at times lack these organs entirely, the female plants almost never produce archegonia. Indeed, in only one polycladous female clone (21.56, belonging to the family here under discussion) have archegonia as yet been found on dissection, and in this clone only in a very few instances. Structures certainly to be recognized as involucres, more or less similar to those borne by typical female plants, are likewise rare. On the other hand, polycladous female plants bear, in variable but sometimes rather large numbers, dorsal structures of a solid columnar form, each composed of several rows of cells. These upgrowths are straight or curved, occasionally forked toward the apex, and fairly often distally expanded or flaring. Three such expanded or vase-like upgrowths are shown near the point marked "a," figure 11. While the columnar upgrowths are often abundant on polycladous female plants, similar structures (and those of doubtful correspondence) rarely appear on polycladous males. The upgrowths in question borne by the polycladous females are interpreted as representing archegonial involucres, their distorted form being an expression of the same genotypic tendency that results in the appearance of reduced or variously atypical antheridial involucres on the male plants. The not infrequent vase-like form is perhaps an indication of a tendency toward the hollow tubular character of typical archegonial involucres.

Clone 21.56, the only one in which archegonia have been observed, is also distinguished from other polycladous females by the fact that at times it has borne fairly numerous—though never abundant—involucres which, while mostly somewhat aberrant, were comparable with those so abundantly borne by typical females. Figures 9 and 10 represent plants of this clone. At b, figure 9, is shown an archegonial involucre of quite aberrant form, and at c and d, figure 10, are two of more nearly typical appearance. At e, figure 7, is shown one of the approximately typical involucres which have appeared with extreme rarity in a few other polycladous female clones.

Clone 21.153 (♀) has been the subject of some doubt. At the times of the earlier observations, this clone displayed a larger proportion of aberrant structures than ordinarily characterizes a typical clone. One branch was noted as "approaching the tufted form," and there was much variation in size and shape of involucres as well as of lateral lobes. It was therefore suspected of being either tufted (differently from either f_1 ancestor) or possessing some tendency to polyclady. This is the clone classed as "doubtful" in the first table of the abstract (ALLEN 1923). At later times, however, this clone appeared typical, and it is classed in

table 1 of the present paper as "possibly atypical." From a later mating of this clone with a typical male, all the offspring obtained were typical or "possibly atypical." Clone 21.153, therefore, possesses no hereditary tendency toward tuftedness or polyclady. Incidentally, it is suggested that the offspring of various matings classed in this and a previous paper (ALLEN 1924) as "possibly atypical" may not differ genotypically from strictly typical clones.

Numerous attempts to secure the fertilization of polycladous females by males of various types have been uniformly unsuccessful. This is true even of clone 21.56, with which eleven such attempts have been made. Inadvertently, a similar experiment was performed on a large scale. A Wardian case, containing about 120 cultures of both sexes and of various types, became flooded during the night by the stoppage of an outlet pipe. In the morning, water was standing in the case above the levels of the pots. Abundant sporophytes appeared later in the cultures of typical and tufted females in this case, but none in any of those of polycladous females including three cultures of clone 21.56. It may be concluded, therefore, that the tendency which results in a partial sterility of the polycladous males, in the sense that the production of antheridia is materially less than in typical races, expresses itself in their polycladous female progeny in the almost complete absence of archegonia and in a total functional sterility. On the other hand, the non-polycladous offspring of polycladous males, both females and males, are uniformly fertile.

A question may be raised as to the propriety of classing these sterile polycladous plants as female. The reasons for so doing are, briefly, that they differ in size from male plants in about the same proportions as do typical females; that their columnar upgrowths seem to represent involucres; and that they possess the same chromosome complement, including the X chromosome, as do typical females.

The determination of the sex of a polycladous clone can be made at most times with reasonable certainty, though not always with the assurance possible in the case of typical or of tufted clones. In general, as already noted, male and female polycladous plants differ in size, but the range of variation in this respect is sufficient so that determinations based upon vegetative characters alone are not always satisfactory. Antheridia are of course conclusive evidence of maleness; but their absence is not proof of femaleness, since male clones are not infrequently, and even for long periods, sterile. The presence of columnar upgrowths in considerable numbers renders it fairly certain that the clone bearing them is female,

but at times these structures are lacking in a female clone, and, on the other hand, apparently similar dorsal upgrowths are sometimes, though rarely, observed on certainly male plants. In consequence of these facts, the possibility of an occasional erroneous determination can be excluded only by repeated observations over an extended period; in which case it is reasonably certain that all the male clones will at one time or another have borne antheridia.

On the other hand, the polycladous habit is so distinctive that there is no difficulty in distinguishing, in the case of plants that have grown to the extent of producing a few branches, between polycladous forms on the one hand and typical or tufted forms on the other. The situation is thus quite different from that confronting one who is dealing with the progeny of crosses between tufted and typical races; in the latter case, sex is determinable very early, but the distinction between the typical and the tufted habit involves observations extended over a considerable period, and even then the possibility of error is not entirely eliminated.

Tufted ♀ × *polycladous* ♂

Mating 14. R35G1×R19δ1aA

Fertilized January 17, 1920. Tetrads sown March 29, 30. Sporelings transplanted June 26, 1920, to January 26, 1921.

The maternal parent in this cross was the tufted clone described in a previous paper (ALLEN 1924) and used in matings 2 to 5 therein reported.

The results of the present mating are shown in table 2. The tetrads from capsules A and B were sown individually, those from capsule C broadcast. The classification of the f_1 progeny may be left for discussion in connection with the results of the mating next to be reported. Three clones resulting from the present mating, however, require special mention.

Clone 20.179 was noted on four occasions as having all the characteristics of a polycladous female, including size and general habit and (on two occasions) the presence of columnar upgrowths. At one time, however, what was taken for an exposed antheridium was seen, as well as were several smaller bodies thought to be young antheridia. In view of the fact that previous and later observations agreed in classing this clone as female, it may be suspected either that the one observation showing it apparently a male was erroneous, or that it indicated an accidental contamination of the culture. Another possibility suggested is that of hermaphroditism—which, however, is supported by no observation on other clones of any type. This particular clone was derived from a spore of the

TABLE 2

(p_1) R35G1 (♀ tufted) × R19δ1aA (♂ polycladous).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	TOTAL NON-TUFTED NON-POLYCLADOUS	CLASS 3 LESS THAN 10 PER-CENT TUFTED	CLASS 4 10 TO 50 PER-CENT TUFTED	CLASS 5 50 PER-CENT OR MORE TUFTED	TOTAL TUFTED NON-POLYCLADOUS	TOTAL NON-POLYCLADOUS	CLASS 6 POLYCLADOUS	TOTAL ALL CLASSES
Capsule A (F_1)										
f_1♀'s	3	0	3	0	0	1	1	5*	1	6
f_1♂'s	0	0	0	0	0	0	0	0	0	0
Total	3	0	3	0	0	1	1	5	1	6
Capsule B (F_1)										
f_1♀'s	1	0	1	0	1	0	1	2	2	4
f_1♂'s	0	0	0	0	0	0	0	0	0	0
Total	1	0	1	0	1	0	1	2	2	4
Capsule C (F_1)										
f_1♀'s	1	3	4	4	2	0	6	10	6	16
f_1♂'s	6	0	6	0	0	3	3	9	7	16
Total	7	3	10	4	2	3	9	19	13	32
Total f_1♀'s	5	3	8	4	3	1	8	17	9	26
Total f_1♂'s	6	0	6	0	0	3	3	9	7	16
Grand total	11	3	14	4	3	4	11	26	16	42

Sex and character not determined: capsule A, 8; capsule B, 1; capsule C, 2; total, 11.
* Includes 1 non-polycladous ♀, not determined as to tuftedness or non-tuftedness.

only tetrad in this family that supplied more than one clone which grew to a sufficient size for satisfactory determination. From the spores of this tetrad came clones 20.178 (polycladous ♀), 20.179 (here under discussion), 20.180 (which died before determination), and 20.181 (polycladous ♀). However, it was noted at the time of transplanting the sporelings, that 20.178 and 20.179 were possibly parts of a single plant and that 20.180 and 20.181 also perhaps belonged together. If 20.179 were really male, it must have come from a spore other than that giving rise to 20.178. It

would follow that at least three spores of the tetrad gave rise to polycladous clones—a condition inconsistent with more recent results in cultures now under observation. In the classification of table 2, therefore, cultures 20.178 and 20.179 are considered as belonging to a single polycladous female clone.

One male clone (20.255) has at times seemed to diverge from the usual polycladous habit, approaching in its vegetative structure somewhat more closely the typical form, although at other times it has appeared strictly polycladous. It is classed as polycladous, with the reservation that it may prove genetically distinct.

Another male clone (21.16) has shown all the characteristics of a tufted race, but at times the aberrancies of its vegetative structures have been so marked as to give rise to the suspicion that it might represent a combination of tufted and polycladous characteristics. However, matings of this clone with typical females have given typical and tufted, but no polycladous, f_1 progeny. 21.16 is therefore referred to class 5, having shown an average tuftedness of about 75 percent.

Mating 15. R27E×R19♂1aB

Fertilized January 24, 1921. ♀ plants with mature sporophytes dried April 12. Spores (not united in tetrads) sown October 25 and 31. Sporelings transplanted January 7 to April 25, 1922.

The maternal plant in this mating was the tufted clone used in matings 8 to 10 previously reported (ALLEN 1924). The results of the germination of the spores from two capsules, sown broadcast, are shown in table 3. From the spores from a third capsule, 24 sporelings were obtained, of which 18 developed into clones large enough to allow the determination of their character. Among them were typical and tufted, but no polycladous clones. The absence of the polycladous class suggested a doubt as to the paternity of the sporophyte from which these spores came,—involving the possibility of a confusion in records or of an accidental fertilization by an antherozoid from a non-polycladous clone. Because of this doubt, the clones derived from the spores of this particular capsule are omitted from the tabulation.

The sex of one f_1 polycladous clone (22.140, classed as a female) is open to question. It was unhealthy because of contamination with blue-green algae and died while still small, after only two observations. No antheridia were seen, nor were columnar upgrowths certainly recognized. Otherwise, the classification given in table 3 is made with reasonable confidence.

TABLE 3

(f_1) *R27E* (♀ *tufted*) × *R19δ1aB* (♂ *polycladous*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	TOTAL NON-TUFTED NON-POLYCLADOUS	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10 TO 50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	TOTAL TUFTED NON-POLYCLADOUS	TOTAL NON-POLYCLADOUS	CLASS 6 POLYCLADOUS	TOTAL ALL CLASSES
Capsule A (F_1)										
f_1 ♀'s	12	1	13	2	6	11	19	33*	30	63
f_1 ♂'s	11	1	12	1	5	2	8	29†	13	42
(Sex ?)									3	3
Total	23	2	25	3	11	13	27	62	46	108
Capsule B (F_1)										
f_1 ♀'s	1	0	1	0	0	0	0	1	0	1
f_1 ♂'s	0	0	0	0	0	0	0	0	0	0
Total	1	0	1	0	0	0	0	1	0	1
Total f_1 ♀'s	13	1	14	2	6	11	19	34	30	64
Total f_1 ♂'s	11	1	12	1	5	2	8	29	13	42
(Sex ?)									3	3
Grand total	24	2	26	3	11	13	27	63	46	109

Sex and character not determined: capsule A, 28.

* Includes 1 non-polycladous ♀, not determined as to tuftedness or non-tuftedness.

† Includes 9 non-polycladous ♂'s, not determined as to tuftedness or non-tuftedness.

The f_1 progeny of this as of mating 14 includes, in both sexes, typical, tufted, and polycladous clones. Among the tufted clones appear the same variations in degrees of tuftedness, and much the same distribution of classes based on these variations, that occurred in the progeny of tufted × typical as previously reported (ALLEN 1924). The class that might have been expected, but does not appear, is that showing a combination of tufted and polycladous characters. The apparent absence of this class is provisionally explained by supposing that the aberrancies due to the inheritance of tuftedness are masked by the greater and uniformly present aberrancies of polyclady. This would imply that some (presumably about half) of the polycladous clones are genotypically both tufted and

polycladous. The sterility of the polycladous females renders a test of this hypothesis by their means impossible; but, if the hypothesis is sound, some of the f_1 polycladous males, mated with typical females, would be expected to give typical, tufted, and polycladous, while others, similarly mated, would yield only typical and polycladous progeny. Experiments of this nature are now under way.

The hypothesis just outlined also leads to the expectation that, among the f_1 progeny of the present and the previous mating, the total numbers of non-polycladous and of polycladous f_1 clones would be about equal. Actually, the results do not quite agree with this expectation. Among the f_1 progeny of mating 14 (table 2) are 17 non-polycladous and 9 polycladous females, 9 non-polycladous and 7 polycladous males; and among those of mating 15 (table 3), 34 non-polycladous and 30 polycladous females, 29 non-polycladous and 13 polycladous males (plus 3 polycladous clones of unknown sex). The discrepancies, however, are of the same order as those resulting from mating 13 between a typical female and a polycladous male, the progeny in that case (table 1) including 46 non-polycladous and 28 polycladous females, 28 non-polycladous and 21 polycladous males (plus 3 polycladous clones of unknown sex). Evidently the lesser number of polycladous progeny from matings 14 and 15 is not to be accounted for by the absence of a tufted+polycladous class. The explanation of this discrepancy must apply equally to mating 13, in which no such class would be expected. The hypothesis remains valid, therefore, that the polycladous clones resulting from matings 14 and 15 include some which have, and some which have not, inherited the tufted tendency.

The proportions of the non-polycladous and polycladous classes are better shown in table 4. Summing all the results of the matings here reported, it appears that in the f_1 generation there were 163 non-polycladous and 114 polycladous clones of both sexes. This numerical discrepancy, appearing in both sexes among the progeny of each mating, might conceivably be accounted for in one of three ways:

(a) That more than half the spores produced by an F_1 sporophyte resulting from a mating of non-polycladous $\times$ polycladous will, if they germinate, give rise to non-polycladous clones. This, stated in Mendelian terms, implies that the difference between polyclady and non-polyclady is determined by at least two pairs of factors, both (or more than one) of the factors for polyclady being necessary to the appearance of that character.

(b) That spores carrying the determiner or determiners for polyclady germinate in smaller proportions than do those giving rise to non-polycladous plants.

TABLE 4

Summary of matings 12 and 13 (♀ typical × ♂ polycladous), and 14 and 15 (♀ tufted × ♂ polycladous).

	NON-POLYCLADOUS	POLYCLADOUS	TOTAL
Mating 12			
f_1 ♀'s	0	0	0
f_1 ♂'s	1	0	1
Mating 13			
f_1 ♀'s	46	28	74
f_1 ♂'s	28	21	49
(Sex ?)		3	3
Mating 14			
f_1 ♀'s	17	9	26
f_1 ♂'s	9	7	16
Mating 15			
f_1 ♀'s	34	30	64
f_1 ♂'s	29	13	42
(Sex ?)		3	3
Total f_1 ♀'s	97	67	164
Total f_1 ♂'s	66	41	107
(Sex ?)		6	6
Total classified	163	114	277
Sex and character not determined			50
Grand total			327

(c) That polycladous plants are less vigorous and die while young in larger proportions than do non-polycladous plants.

Supposition (c) seems to be negatived by the observation that polycladous plants grow at least as vigorously as do typical or tufted plants of the same sex. Indeed, polycladous males often seem to be more resistant to unfavorable conditions, and to grow more rapidly, than typical or tufted males. On suppositions (a) and (b), present information throws no light. Both may be tested, however, if a fuller germination of the spores of isolated tetrads can be secured; and experiments now under way promise results more satisfactory in this respect.

SUMMARY

1. The character (or character complex) designated as "polycladous" was first observed in two male plants of *Sphaerocarpos Donnellii*, probably of common origin.

2. Polycladous plants are uniformly distinguished from typical plants of the same species by marked aberrancies in vegetative structure as well as in the development and the form of involucres. Antheridia are always comparatively few and at times entirely lacking in polycladous males. Archegonia are very rare in polycladous females, and, so far as experiments to the present time have shown, their eggs are entirely functionless.

3. A typical female mated with a polycladous male gives non-polycladous and polycladous f_1 progeny of each sex.

4. The results of a mating of a tufted female with a polycladous male are similar, except that among the non-polycladous f_1 progeny in either sex occurs the same range from typical to highly tufted that appears among the progeny of a tufted female mated with a typical male.

5. Among the f_1 progeny of matings of both the types above-mentioned, the number of polycladous clones in either sex appears to be regularly somewhat smaller than the number of non-polycladous clones. The explanation of the discrepancy must be supplied by future study.

LITERATURE CITED

ALLEN, C. E., 1919 The basis of sex inheritance in Sphaerocarpos. Proc. Amer. Philos. Soc. **58**: 289-316. *28 text figs.*

1923 The transmission of the polycladous character in *Sphaerocarpos Donnellii*. (Abstract), Anat. Rec. **24**: 407.

1924 Gametophytic inheritance in Sphaerocarpos. I. Intraclonal variation, and the inheritance of the tufted character. Genetics **9**: 530-587. *60 text figs.*

THE LINKAGE OF DARK-EYE AND COLOR IN MICE[1]

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INTRODUCTION

DARBISHIRE'S (1904) data on a cross between pink-eyed colored waltzers and albino mice suggested a possible linkage between the genes for color and dark-eye, and prompted a reinvestigation of their relationship. HALDANE, SPRUNT and HALDANE (1915) repeated that part of DARBISHIRE'S work dealing with these genes, and concluded that there was a gametic ratio of about 3 : 1, suggesting approximately 25 percent crossing over. Their tentative conclusion was based on very small numbers, however, and largely from matings of hybrids *inter se*. CASTLE (1919) and DUNN (1920) began a series of experiments on a larger scale in 1916, in which the F_1 dihybrids were mated back to the double recessive,—a more advantageous type of mating. More recently CASTLE and WACHTER (1924) recorded additional data on this cross, and DETLEFSEN and CLEMENTE (1924) reported on a cross involving similar genes (color-dilution, allelomorphic to color, was substituted for color). The writer had begun similar experiments with the genes for color and dark-eye in 1917 without being aware of contemporary investigations on these genes. The present paper is a report on these experiments, and a summary of all similar data in previous investigations. A revision of the method of pooling such data, and calculating the variability is suggested; for, in general, it has seemed to me that the probable errors of crossover values, estimated in the manner necessary in these data, have been too low.

[1] The data were collected at the College of Agriculture, UNIVERSITY OF ILLINOIS. The writer is indebted to Doctor E. ROBERTS for material aid in recording the classifications and pedigrees in these experiments.

My original crosses between pink-eyed colored (*Cd Cd*) and albinos carrying dark-eye (*cD cD*) gave F_1 dark-eyed colored hybrids (*Cd cD*), which were at first mated *inter se*, primarily to secure a stock of double recessives (*cd cd*), since backcrossing the F_1 hybrids to double recessives was obviously preferable to mating them *inter se*.[2] Incidentally these matings also gave data on linkage which are included for the sake of completeness.

TABLE 1

The distributions of offspring from dihybrids (Cd cD) mated inter se. Classified after pelage could be identified.

SOURCE OF DATA	DARK-EYED COLORED	PINK-EYED COLORED	ALBINO	TOTAL	ON A 9:3:4 BASIS	
					χ^2	P
DARBISHIRE 1904	284	134	137	555	11.180	0.0038
DUNN 1920	295	129	156	580	7.598	0.0231
New data	390	159	216	765	8.668	0.0135
Total, observed	969	422	509	1900		
Expected, gametic ratio, 1 : 1	*1068.75*	*356.25*	*475*	*1900*	*23.879*	*0.0000*
Expected, gametic ratio, 6 : 1	*959.69*	*465.31*	*475*	*1900*	*6.554*	*0.0389*

These original data accumulated in process of establishing the stock of double recessives, together with similar data from other sources, are given in table 1. All individuals in this table represent the young classified after the pelage could be identified, and may therefore represent the residues left after variable amounts of selective elimination. They are subject to this criticism. If the genes for color and pink-eye are independent, the dihybrids (*Cd cD*) should give a 9 : 3 : 4 ratio in the F_2. Neither DARBISHIRE's nor DUNN's, nor my data gave distributions which agree even reasonably well with the distributions expected on the basis of independence; and the total results from all these experiments agree very poorly with a gametic ratio of 1 : 1. If we make the total results with 1900 offspring a test against a distribution based on a 6 : 1 gametic ratio (since 14.29 percent crossing over is approximately the value found in the more reliable birth records) the observed results and the theoretical are closer; but even here a value of $P = 0.0389$ is lower than one might reasonably expect if the observed deviations are due to random sampling.

[2] Professor W. E. CASTLE had already established this stock, and generously supplied the writer with an abundance of material in 1918, when we discovered we were investigating a similar problem.

It is futile to apply fastidious precision to these data, since any one of a number of possible gametic ratios is not excluded, and no gametic ratio can give a perfect fit. In testing the total results of these experiments against *any* gametic ratio, the albinos will always constitute theoretically 25 percent of the total, will always deviate by 34 (509 − 475), and will therefore always contribute a constant to χ^2 accordingly. The sum of the deviations of the remaining classes must also equal 34, since we are dealing with a correlated system of variables and it simply remains to find for these remaining classes a set of calculated values which will contribute least to χ^2. A gametic ratio of about 2.34 : 1 (or 29.98 percent crossing over) will satisfy these conditions, for with it P = 0.2016. Now this crossover value is quite different from that which CASTLE and DUNN found in backcrosses and also from similar backcross data recorded in this paper, in which the crossover values are likewise based on birth records. Evidently the crossover values observed in mice are subject to much distortion, and the events taking place between birth and the production of the pelage may play a part. If a gametic ratio of 6 : 1 is approximately correct for a birth classification (I am disregarding the question of any distortion due to prenatal elimination) the elimination of the pink-eyed colored during the first few weeks of postnatal life is relatively the most severe, as table 1 shows.

More reliable conclusions can be drawn from the backcrosses of the dihybrids to the double recessive. In CASTLE'S, in DUNN'S and in our experiments, the presumptive crossovers in the backcrosses were estimated directly from the proportion of dark-eyed young in the birth records. In the absence of any disturbing conditions such as prenatal elimination, the dark-eyed young in the repulsion experiments represent one-half of the recombinations, and in the coupling experiments they represent one-half of the parental combinations. From the dark-eyed colored young at birth one may then estimate the probable number of crossovers, since all young showing pigmented eyes carry the color factor. If prenatal elimination of the pink-eyed forms takes place in the repulsion series, it tends to increase the crossover value; but if it takes place in the coupling series, it tends to decrease the crossover value.

REPULSION

In the repulsion experiments, heterozygous males and females, $\frac{C\ d}{c\ D}$, were mated to the double recessive, $\frac{c\ d}{c\ d}$, producing four genotypes as follows:

TABLE 2

Repulsion. Distribution of F_2 repulsion offspring from F_1 Cd cD (P_1=CCdd × ccDD[3]).

SOURCE OF DATA	BIRTH RECORD					CLASSIFIED AT ABOUT TWO WEEKS				
	Dark-eyed	Pink-eyed	Total	Estimated crossovers	Percent of estimated crossovers	Dark-eyed colored	Pink-eyed colored	Albinos	Total	$\frac{2\,C\,D}{\text{Total}}$
F_1 ♂♂	24	520	544	48	8.82±1.19	20	172	188	380	10.526
F_1 ♀♀	173	1827	2000	346	17.30±0.85	125	558	819	1502	16.644
Total	197	2347	2544	394	15.49±0.71	145	730	1007	1882	15.409

TABLE 3

Coupling. Distribution of F_2 offspring from F_1 C D c d (P_1=CC DD × cc dd).

SOURCE OF DATA	BIRTH RECORD					CLASSIFIED AT ABOUT TWO WEEKS				
	Dark-eyed	Pink-eyed	Total	Estimated crossovers	Percent of estimated crossovers	Dark-eyed colored	Pink-eyed colored	Albinos	Total	$\frac{2\,C\,d}{\text{Total}}$
F_1 ♂♂	342	387	729	45	6.17±2.49	186	19	190	395	9.620
F_2 ♂♂	121	132	253	11	4.35±4.24	86	12	77	175	13.714
Total ♂♂	463	519	982	56	5.70±2.15	272	31	267	570	10.877
F_1 ♀♀	112	106	218	−6	−2.75±4.57	83	7	66	156	8.974
F_2 ♀♀	189	237	426	48	11.27±3.25	119	19	133	271	14.022
F_3 ♀♀	143	195	338	52	15.38±3.62	73	11	99	183	12.022
Total ♀♀	444	538	982	94	9.57±2.14	275	37	298	610	12.131
Grand total	907	1057	1964	150	7.64±1.52	547	68	565	1180	11.525

[3] The probable errors of this and other primary distributions were calculated from the formula I suggested in a former paper (DETLEFSEN and CLEMENTE 1924). It seemed that some revision was necessary when the number of crossovers was inferred from a single crossover class or single parental class.

C d	*c D*	*c d*	*C D*
Pink-eye color	Dark-eye albinism	Pink-eye albinism	Dark-eye color
Parental types		Crossovers	

All pregnant females were placed in isolation pens to avoid crowding and competition, and to give the optimum conditions as far as possible. Pregnancy pens were examined daily, so that the young were recorded within 24 hours of birth. The young at birth fell into two distinct groups, the pink-eyed and the dark-eyed. The former included the pink-eyed colored and all albinos; the latter consisted of the dark-eyed colored alone, theoretically one-half the crossovers. As soon as the pelage could be accurately determined (at about two weeks) a second classification was made. The results of both classifications are given in table 2.

In general, the crossover values as determined from our birth records agree more closely with those which CASTLE (1919) and DUNN (1920) found in their birth records than with the value derived by HALDANE and his co-workers (1915), or with the value indicated by the offspring from F_1 hybrids mated *inter se* and shown in table 1. The outstanding fact is the low value shown by the F_1 males, a value which at first might imply an unquestionable difference when compared with the F_1 females, for the difference is 8.48 ± 1.46 percent and $\frac{\text{Dev.}}{\text{P. E.}} = 5.81$. While the difference appears to be significant in a statistical sense, if we resort to a routine determination of probable errors, it can not immediately decide the important question of a functional difference in spermatogenesis and oögenesis in mice, relative to crossing over. We may eliminate differential viability as a cause of this low crossover value shown by males in the repulsion series; for the survival value of the dark-eyed is certainly not less than that of the pink-eyed, and therefore the net effect would be to *raise* the crossover value if any change in crossover value is to be attributed to prenatal selection. Furthermore, the average numbers of young per litter from F_1 males (4.57 based on 119 litters) and from F_1 females (4.76 based on 420 litters) suggest no sufficiently well-marked difference in prenatal competition and elimination. The chief objection to any far-reaching conclusions as to sex differences in crossing over, *based on these data alone*, lies in the nature of the distribution from which we purport to calculate the variability. In these linkage studies we have no *a priori* probabilities based on a logical theory, as in simple Mendelism,

against which we can test our results; and we simply assume that the observed proportion of dark-eyed is sufficiently accurate for practical purposes. However, we cannot overlook the possibility that this low value may represent only a reasonably wide, but possible, deviation from some higher value as a "true" value. Let us assume that 7.14 percent is the "true" proportion of dark-eyed and 14.29 percent is the corresponding crossover value,—about the average value for both sexes combined. The calculated number of dark-eyed would be 38.86 ± 4.05. The observed number of dark-eyed (24) is then by no means so low as to justify a conclusion that the males are certainly functionally different from the females in crossing over. Furthermore our curve of random fluctuations is, if anything, slightly asymmetrical. This simply means that any stipulated reasonable limits of error include more random deviations below the calculated mean crossover value than above, the exact proportions depending on the shape of the curve. Therefore, we may expect to find variation downward perhaps a little more frequently than upward. In view of these facts, I hesitate to place any great confidence in the apparent difference of 8.48 percent in crossing over between males and females,—at least as far as this individual experiment in itself is concerned. The question of combining it with other similar results, I shall discuss later in this paper.

The more complete classification at the appearance of the pelage gave crossover results (table 1) which on the whole are of about the same magnitude as those shown by the birth records. There is some evidence of distortion in the pink-eyed classes, for the total albinos (1007) should equal the total colored (875). There is, then, a very suggestive deviation from the theoretical value (941) for each class. The pink-eyed colored have apparently undergone greater elimination than their complementary parental class, the albinos carrying dark-eye (a larger proportion of the albinos carry dark-eye, since this is a parental class).

In addition to classifying the offspring when about two weeks old, 125 albinos were chosen at random and tested to see whether they were *cD* or *cd*. In mating them to pink-eyed colored, the production of a single dark-eyed would immediately determine the zygotic constitution as *cD*. Where no dark-eyed were produced in 14 or more offspring, we may decide with reasonable safety that the tested albino is *cd*; for the probability that an albino carrying dark-eye would produce only pink-eyed young in 14 offspring from such a mating is $\frac{1}{16{,}384}$. Among these 125 albinos, 113 (45♂♂+68♀♀) proved to be *cD*, and 12 (9♂♂+3♀♀) proved

to be *cd*, when tested as rigidly or more rigidly than indicated above. There were, then, 10.42 percent crossovers among the albinos, as classified after sex maturity.

COUPLING

In the coupling experiments, the heterozygous males and females, $\frac{C\ D}{c\ d}$, were mated to double recessives, $\frac{c\ d}{c\ d}$, producing four genotypes, as follows:

$\frac{C\ D}{}$	$\frac{c\ d}{}$	$\frac{c\ D}{}$	$\frac{C\ d}{}$
Dark-eye color	Pink-eye albinism	Dark-eye albinism	Pink-eye color
Parental types		Crossovers	

The routine procedure of care, isolation of pregnant females, and classification of young was the same as in the repulsion series. The young fell into two groups at birth, the dark-eyed and the pink-eyed. The former represented one-half of the parental types. The number of crossovers is inferred from the difference between the pink-eyed and dark-eyed at birth, which is precisely the same as doubling the dark-eyed and subtracting this from the total. At the appearance of the pelage a second classification was made. The results of both classifications are given in table 3.

Table 3 shows two outstanding facts,—the wide fluctuations in crossover values, and their small average magnitude. The great variability is not unexpected, for, entirely apart from other causes of variability, the purely random fluctuations in estimated percentage of crossovers will be twice as great as in the percentage of dark-eyed present, as has been explained in an earlier paper (DETLEFSEN and CLEMENTE 1924). The low crossover values agree in a suggestive way with those found for coupling of color dilution and dark-eye (DETLEFSEN and CLEMENTE 1924). Comparing them we find:

Color versus dark-eye	*Color dilution versus dark-eye*
♂♂ = 5.70±2.15 percent	♂♂ = 5.73±2.55 percent
♀♀ = 9.57±2.14 percent	♀♀ = 11.62±3.20 percent

It would seem that there is more evidence of elimination in the coupling series than in the repulsion, and the reason may lie in the widely different proportion of double recessives in the two types of mating. In repulsion,

the double recessives form at most about one-fourteenth of the total, while in coupling they form about six-fourteenths of the total. That which survives in competition is of course a relative, but not necessarily a simple matter in which several antecedent factors, both genetic and non-genetic may enter. The lowest value is found in the male coupling series, and in this series we have the combination of two circumstances unfavorable for the survival of offspring: (1) double recessives used as dams; and (2) about three-sevenths of the zygotes formed are likewise double recessives. In the female coupling series we used the vigorous hybrids as dams. It may therefore be pertinent to the problem to point out that the smallest average per litter and lowest crossover value are found in the male coupling series (table 3), where we might logically expect the greatest elimination; while the largest average per litter and highest crossover value are found in the female repulsion series (table 2), where conditions are the most favorable, since the dams were vigorous hybrids and the double recessives composed only a small fraction of the total offspring. The data have been rearranged in table 4 to put this thought in simple comprehensible form. The main point is that two conceivably important contributory causes of elimination go with the male coupling series, while two contrasted causes conducive to survival go with the female repulsion series. Vigor of dams and offspring tend to balance each other in the female coupling and male repulsion series, and they are intermediate in litter size. If our interpretation is correct, the net effect would be to lower the crossover values of the male and female coupling series by reducing the number of pink-eyed; but to raise the crossover values in the male and female repulsion series or leave it unmodified, according to the amount of elimination of pink-eyed. I should not care to insist or even imply that *all* the difference between males and females in this coupling cross was due solely to selective elimination; not yet do I care to inject a functional difference in spermatogenesis and oögensis into the conclusions without first exhausting other possibilities. In any event, it is only too easy to lapse into specious reasoning, for the non-genetic factors are complicated. For example, vigorous dams and a small fraction of double-recessive offspring in the female repulsion series may of themselves imply less prenatal elimination, but at the same time these antecedents may carry with them greater fecundity and therefore lead to some competition after all.

It is noteworthy that the males in table 2 and both sexes in table 3 give absolute values much lower than those found by either DUNN or CASTLE and WACHTER. The mechanism responsible for Mendelian re-

TABLE 4

A comparison of litter averages and percentages of crossing over, in various forms of mating in mice, and in relation to the elimination of offspring.

SOURCE OF DATA	NUMBER OF LITTERS	TOTAL OFFSPRING	AVERAGE PER LITTER	PERCENTAGE OF ESTIMATED CROSSOVERS	CONDUCIVE TO SURVIVAL OF OFFSPRING	
					Vigor of dams	Vigor of offspring
♂ Coupling	231	982	4.25	5.70±2.15	−	−
♀ Coupling	223	982	4.40	9.57±2.14	+	−
♂ Repulsion	119	544	4.57	8.82±1.19	−	+
♀ Repulsion	420	2000	4.76	17.30±0.85	+	+
Total	993	4508	4.54	12.07±0.77		

TABLE 5

The standard errors in coupling and in repulsion series, where the crossovers are estimated on the basis of a single parental class (coupling) or a single crossover class (repulsion). A method of treating the combined series is suggested. Any standard error may be converted directly into probable error.

TYPE OF MATING	OBSERVED DARK-EYED	OBSERVED DARK-EYED	TOTAL	PERCENTAGE OF DARK-EYED	ESTIMATED CROSSOVERS	PERCENTAGE OF ESTIMATED CROSSOVERS
Coupling	$m \pm \sigma_1$	$n \pm \sigma_1$	s	$100\frac{m}{s} \pm 100\ \frac{\sigma_1}{s}$	$n-m \pm 2\sigma_1$	$100\frac{n-m}{s} \pm 200\frac{\sigma_1}{s}$
Repulsion	$p \pm \sigma_2$	$q \pm \sigma_2$	t	$100\frac{p}{t} \pm 100\frac{\sigma_2}{t}$	$2p \pm 2\sigma_2$	$100\frac{2p}{t} \pm 200\frac{\sigma_2}{t}$
Total, both series	$(m+p) \pm \sqrt{\sigma_1^2+\sigma_2^2}$	$(n+q) \pm \sqrt{\sigma_1^2+\sigma_2^2}$	$s+t$	$100\frac{m+p}{s+t} \pm \frac{100}{s+t}\sqrt{\sigma_1^2+\sigma_2^2}$	$(n-m+2p) \pm 2\sqrt{\sigma_1^2+\sigma_2^2}$	$100\frac{n-m+2p}{s+t} \pm \frac{200}{s+t}\sqrt{\sigma_1^2+\sigma_2^2}$

combinations in mice is of a relatively precise nature (as the variability in Mendelian ratios in mice indicates (DETLEFSEN 1916). The non-Mendelian recombinations in mice seem to lack this constancy, and the mechanism is, perhaps, more readily subject to genetic and non-genetic disturbances. It has been shown in Drosophila that variability in crossover values is simply enormous (GOWEN 1919), and marked changes have been shown to take place easily in one chromosome at least (DETLEFSEN 1919). It should also be stated that the coupling values found by DUNN in mice were not lower than the repulsion, and therefore do not support the special explanation I found necessary to apply to my results in table 4. However, CASTLE's values found in the linkage of red-eye and pink-eye in rats occur in exactly the same order of magnitude, the male and female coupling values and the male and female repulsion values occurring in the order stated: 13.60, 19.89, 18.81, 21.54. There is a difference of 7.94 percent between male coupling and female repulsion in CASTLE's rat series, and a difference of 11.60 percent between corresponding values in my mouse series. If I were to hazard a judgment without certain knowledge, I should say that in CASTLE's rat series and my mouse series the male coupling values should be raised most, and the female coupling values somewhat; but the male repulsion values should perhaps be lowered. This suggestion has reference to the effects of elimination alone, which effects may be superimposed on any reasonable amount of random fluctuation in sampling of zygotes in a plus or minus direction.

The complete classification of offspring in the coupling series at the end of two weeks is given in table 3. If we calculate the percentage of crossovers in the same way as in the birth records (subtracting dark-eyed from pink-eyed) the values remain quite as low; but if we estimate the crossovers by doubling the single visible crossover class (the pink-eyed colored) the crossover value is, in general, raised. The presumptive evidence shows that the albinos are too few. Now, the albinos include two classes, (1) *c d*, the double recessive parental type, and (2) *c D*, the crossovers. Among the offspring of the males the total albinos are actually less frequent than the single parental dark-eyed class. It is impossible to know without a long and tedious genetic test of the albinos whether this paucity is due to a greater relative depletion of the *c d* class or the *c D* class. It is noteworthy, that the albinos are more rigidly eliminated among the offspring of the males than among the offspring of the females, which fact agrees with our contention in table 4.

CROSSING OVER IN RELATION TO SEX

In the coupling and in the repulsion series, the males showed a lower absolute crossover value than the females. If we combine the coupling and repulsion series, the same sex difference remains. CASTLE's (1919), DUNN's (1920), CASTLE and WACHTER's (1924), DETLEFSEN and CLEMENTE's (1924) data, and the data described in this paper are all strikingly consistent in this respect. However, we cannot draw an unreserved conclusion that the difference between the sexes really represents a specific functional difference between male and female mice until we determine (1) whether the difference is significant, and (2) if it is significant, that other possible extraneous and non-genetic causes are excluded. DUNN concluded that his males and females differed by 2.26 ± 0.59 percent $\left(\frac{\text{Dev.}}{\text{P. E.}} = 3.81\right)$, apparently very suggestive and certainly very important if substantiated. But it has been shown in an earlier paper that the standard deviations of such crossover values as are estimated on the basis of a single parental class (coupling) or on the basis of a single observed crossover class (repulsion) have been greatly underestimated. Furthermore, in the total complex derived by pooling coupling and repulsion series, the sum of the estimated crossovers has been treated as simple observed crossover frequency without regard to the variability of its components. Since to estimate the crossovers in coupling we subtract the observed dark-eyed from the pink-eyed, and in repulsion we double the observed dark-eyed, and since the random fluctuations of estimated crossover values in coupling and in repulsion are influenced by these methods, it is obvious that neither process can be used alone to estimate the crossovers and the variability in a combined coupling and repulsion series. Therefore, we must first inquire what procedure to follow in dealing with the primary distribution of the separate component series, and then with the combined series. It will be simpler to take up the general case first, and apply it to the experimental data later.

In any coupling experiment with a given sex, let

m = the number of observed dark-eyed,
n = the number of observed pink-eyed, and
$m+n=s$

The random fluctuations in the estimated crossover value depend upon the fluctuations in these observed values. The estimated number of crossovers will be $n-m$. Furthermore, let $\sigma_1 = \sqrt{\frac{mn}{s}}$, the standard error

of the observed number of dark-eyed (or pink-eyed). It follows then that:

$m \pm \sigma_1$ = the number of observed dark-eyed and its standard error.

$(n-m) \pm 2\sigma_1$ = the number of estimated crossovers and its standard error.

$100\frac{m}{s} \pm 100\frac{\sigma_1}{s}$ = percentage of observed dark-eyed and its standard error.

$100\frac{n-m}{s} \pm 200\frac{\sigma_1}{s}$ = percentage of estimated crossovers and its standard error.

In a similar manner, in any repulsion experiment with the same given sex, let

p = the number of observed dark-eyed,
q = the number of observed pink-eyed,
$p+q=t$, and
$\sigma_2 = \sqrt{\frac{pq}{t}}$, the standard error of the observed dark-eyed.

Expressions similar to those of the coupling series may be written down at once. Putting all the foregoing into tabular form, we have the two sorts of distributions which we add together to obtain the total crossover results in a given sex (table 5). In adding the corresponding members of the two series, we can assume that variations in the components are uncorrelated. The total of dark-eyed has no biological significance for us, since the sum is a hotchpot of parental and crossover types, and presents no constant value, but depends entirely upon the relative weight of the coupling and repulsion experiments. The same is true of the percentage of dark-eyed in the total, and it cannot be used as a basis for direct comparison between groups such as males and females. But the percentage of dark-eyed in a single homogeneous series can be used for such purposes just as well as the crossover value itself, giving precisely the same results; as, for example, when comparing males and females in a coupling series (see DETLEFSEN and CLEMENTE 1924). To add the percentages as such would have no meaning for us; and we must first reduce them to a different scale, reducing the errors to the same scale, before we add. This process is, after all, identical with calculating a weighted mean of the percentages and its error. We shall have occasion, in combining all the series from the various investigations, to make use of this general formula. If $M \pm E$ is a

weighted mean and its error, derived from a series of values, $m_1, m_2, \ldots .m_n$ affected by weights $w_1, w_2, \ldots . w_n$ and errors $e_1, e_2, \ldots . e_n$, respectively, then

$$M \pm E = \frac{w_1m_1 + w_2m_2 + \ldots + w_nm_n}{w_1 + w_2 + \ldots\ldots\ldots + w_n}$$

$$\pm \frac{1}{w_1 + w_2 + \ldots + w_n} \sqrt{w_1^2e_1^2 + w_2^2e_2^2 + \ldots + w_n^2e_n^2}$$

Summarizing the results of all linkage experiments in mice, in table 6, in which the errors have been calculated according to the method indicated in table 5 and in the previous discussion, we find that:

1. Not one of the four independent coupling experiments, as it stands, shows a significant difference between the males and females in crossover value, although each one shows a higher absolute value for the females.

2. Combining all the coupling data, a total of 10,312, fails to reveal a significant difference between males and females; for the difference, 2.14 ± 1.40 percent, is only 1.531 times the error of the difference. Since the difference is not significant, it is unnecessary to resort to differential elimination of pink-eyed offspring to explain the lower male value in the total, although the consistent appearance of slightly lower male values in the components may be attributed logically to this cause.

3. DUNN'S repulsion experiment showed no significant difference between males and females. While the data given in this present paper apparently do show a significant difference in this case of repulsion alone, I hesitate to lay stress on this single case for reasons already stated. If we wish to accept unconditionally the values as they stand, the totals in the repulsion data appear to demonstrate a significant difference between males and females; but we must not overlook the fact that this difference in the total repulsion data is largely due to the influence of one experiment (the original data of this paper).

4. There are certain obvious objections to combining repulsion and coupling data (among others the distortion due to elimination of the less viable types is apparent in only a part of the data). If we wish to waive these objections, the grand totals in mice involving 15,997 offspring give male and female crossover values of 12.01 ± 0.63 percent and 15.39 ± 0.66 percent, respectively. The difference, 3.37 ± 0.9 percent, is 3.715 times its error. Here again the difference between males and females in the grand total is due to a single subgroup (the repulsion data of this paper) and we must not overlook this fact if we are to draw a conservative conclusion.

If we examine the totals of all coupling and repulsion data in the individual experiments, the males and females do not show a significant difference in DUNN's total data, and in CASTLE and WACHTER's total data. From my point of view, DUNN's males and females (13.66 ± 0.88 percent and 15.92 ± 1.02 percent, respectively), differ by 2.26 ± 1.35 percent; and $\frac{\text{Dev.}}{\text{P. E.}} = 1.68$; while in CASTLE and WACHTER's totals, the males and females (13.77 ± 0.72 percent and 16.44 ± 0.97 percent, respectively), differ by 2.67 ± 1.21 percent, and $\frac{\text{Dev.}}{\text{P. E.}} = 2.21$. In the totals in my experiments the males and females (6.82 ± 1.45 percent and 14.76 ± 0.91 percent, respectively), differ by 7.94 ± 1.71 percent, and $\frac{\text{Dev.}}{\text{P. E.}} = 4.65$. This large difference is due to the influence of my single repulsion experiment.

On the whole, I am inclined to question the decisive value of all the coupling data in mice and rats because elimination of the weaker classes would of itself favor a lower male crossover value. More conservative and reliable conclusions can be drawn from the repulsion data, because elimination of the weaker classes would favor an increase in the male crossover value, and if the males show a lower value then the females, they do so notwithstanding their handicap.

For the sake of comparing rats and mice, I have included CASTLE's (1919) data and CASTLE and WACHTER's (1924) data in table 6, calculating the errors again as indicated in table 5. In rats, the genes for red-eyed and pink-eyed fail to show a significant sex-difference in crossover value in both coupling and repulsion. If we wish to pool the coupling and repulsion data for each sex, the males and females (15.60 ± 1.28 percent and 20.46 ± 1.13 percent, respectively) differ by 4.90 ± 1.71 percent. The difference is 2.87 times its error, which does not imply significance, as it stands. In the case of repulsion between albinism and pink-eye, the males (18.44 ± 0.27 percent) and the females (21.93 ± 0.39 percent) differ by 3.49 ± 0.48 percent; and $\frac{\text{Dev.}}{\text{P. E.}} = 7.336$. In this case, it seems to me, we have the only evidence of a difference in recombination in males as compared with females, in which the results are clear, significant, and without the objections of a plausible alternative explanation. I have not taken up the case of albinism and red-eye in rats in which the males gave a lower value than the females, because the value of q is some 700 times the value of p, and $q - p$ is rather large compared with $\sqrt{pqn}$.

TABLE 6

A summary of all data on crossing over in mice, in which the sexes are compared. Similar data are given for the rat.

GENES INVOLVED	SEX OF PARENT	TYPE OF MATING	SOURCE OF DATA	OFFSPRING: Dark-eyed	OFFSPRING: Pink-eyed	TOTAL	ESTIMATED CROSSOVERS	PERCENTAGE OF ESTIMATED CROSSOVERS	DIFFERENCE	DIFFERENCE / ERROR
Mice	Male	Coupling	DUNN (1920)	819	1092	1911	273	14.29±1.53	2.91±2.33	1.247
	Female	Coupling		595	825	1420	230	16.20±1.77		
	Male	Coupling	CASTLE and	2250	2985	5235	735	14.04±0.92	2.96±1.76	1.685
	Female	Coupling	WACHTER (1924)	820	1156	1976	336	17.00±1.49		
	Male	Coupling	DETLEFSEN and	329	369	698	40	5.73±2.55	5.89±4.09	1.441
	Female	Coupling	CLEMENTE (1924)	194	245	439	51	11.62±3.20		
	Male	Coupling	This paper	463	519	982	56	5.70±2.15		
	Female	Coupling		444	538	982	94	9.57±2.14	3.87±3.03	1.275
Color *versus* dark-eye	Male	Coupling	Total*	3042	3873	6915	831	12.02±0.81	2.14±1.40	1.531
	Female	Coupling		1458	1939	3397	481	14.16±1.15		
	Male	Repulsion	DUNN (1920)	115	1657	1772	230	12.98±0.79	2.65±1.26	2.109
	Female	Repulsion		107	1262	1369	214	15.63±0.98		
	Male	Repulsion	This paper	24	520	544	48	8.82±1.19	8.48±1.46	5.808
	Female	Repulsion		173	1827	2000	346	17.30±0.85		
	Male	Repulsion	Total	139	2177	2316	278	12.00±0.67	4.62±0.92	4.998
	Female	Repulsion		280	3089	3369	560	16.62±0.64		
	Male	Combined series	Grand total	3181	6050	9231	1109	12.01±0.63	3.37±0.91	3.715
	Female	Combined series	Grand total	1738	5028	6766	1041	15.39±0.66		
Rats	Male	Coupling	CASTLE (1919)	556	731	1287	175	13.60±1.86	6.29±2.44	2.572
	Female	Coupling		699	1046	1745	347	19.89±1.58		
Red-eye *versus* pink-eye	Male	Repulsion	CASTLE (1919)	73	703	776	146	18.81±1.41	2.72±1.96	1.384
	Female	Repulsion		101	837	938	202	21.54±1.36		
Color vs. pink-eye	Male	Repulsion	CASTLE and	1960	19295	21255	3920	18.44±0.27	3.49±0.48	7.336
	Female	Repulsion	WACHTER (1924)	1259	10221	11480	2518	21.93±0.39		

* The totals in DUNN'S experiments are included in CASTLE and WACHTER'S summary; hence, they are not duplicated in my totals.

SUMMARY AND GENERAL CONCLUSIONS

1. Matings of heterozygotes in color and dark-eye (*Cd cD*) *inter se* gave a crossover value of 29.98 percent in 1900 offspring. The offspring were classified after the appearance of the pelage, and the results represent the residue left after selective elimination of the less viable types.

2. In the total results of backcrosses, including both coupling and repulsion data, 4508 offspring gave an estimated crossover value of 12.07 $\pm$0.77 percent. The total repulsion data gave 15.49$\pm$0.71 percent in 2544 offspring, and the total coupling data gave 7.64$\pm$1.54 percent in 1964 offspring.

3. The coupling results were low because selective elimination is more likely to take place in this type of mating, and any decrease in the relative number of pink-eyed forms leads to a lower crossover value.

4. A revision of the methods for calculating variability of crossover values is suggested where such values are estimated on the basis of a single parental class (coupling) or single crossover class (repulsion.) The problem of pooling the results of coupling and repulsion in a given sex, and calculating the variability of the resulting crossover value is considered.

5. There is no evidence of a significant sex-difference in the coupling experiments in mice or rats, either separately or collectively. Even if there were, it is questionable whether such coupling experiments would have any decisive value, since elimination, of itself, favors a lower male crossover value.

6. In the repulsion experiments, a significant sex-difference occurs in only one set of experiments in mice.

The best and practically the only unobjectionable evidence of a case of sex-difference in crossing over in mammals is found in CASTLE'S repulsion data on albinism and pink-eye in rats.

LITERATURE CITED

CASTLE, W. E., 1919 Studies of heredity in rabbits, rats and mice. Carnegie Inst. Washington Publ. 288, 56 pp.

CASTLE, W. E., and WACHTER, W. L., 1924 Variations of linkage in rats and mice. Genetics **9**: 1-12.

DARBISHIRE, A. D., 1904 On the results of crossing Japanese waltzing with albino mice. Biometrika **3**: 1-51.

DETLEFSEN, J. A., 1920 Is crossing over a function of distance? Proc. Nation. Acad. Sci. **6**: 663-670.

DETLEFSEN, J. A., and CLEMENTE, L. S., 1924 Linkage of a dilute color factor and dark-eye in mice. Genetics **9**: 247-260.

DUNN, L. C., 1920 Linkage in mice and rats. Genetics **5**: 325-343.

GOWEN, J. W., 1919 A biometrical study of crossing over. On the mechanism of crossing over in the third chromosome of *Drosophila melanogaster*. Genetics **4**: 205-250.

HALDANE, J. B. S., SPRUNT, A. D., and HALDANE, N. M., 1915 Reduplication in mice. Jour. Genetics **5**: 133-135.

THE INHERITANCE OF STERILITY AND OF OTHER DEFECTS INDUCED BY ABNORMAL FERTILIZATION IN THE PARASITIC WASP, *HABROBRACON JUGLANDIS* (ASHMEAD)

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INTRODUCTION

In 1916 a study of the life history and method of sex determination of the parasitic wasp, *Habrobracon juglandis* (Ashmead), then called *Hadrobracon brevicornis* (Wesmael), was undertaken by P. W. WHITING. By 1918 (WHITING 1918) he had arrived at the conclusion that in this species fertilized eggs produce females and unfertilized eggs produce males. Cytological work then in progress seemed to indicate that the males are haploid and the females diploid as in the honey-bee and hornet. In 1920 an orange-eyed mutant appeared among 253 typical black-eyed brothers, and crosses (WHITING 1921 a) involving this recessive character forced the conclusion that occasional males arise from fertilized eggs and that such males behave in an unusual manner in breeding tests.

In June 1922 the problem concerned with the production of such anomalous males and their behavior in breeding was turned over to me and work on it has been continued up to this time. The results of the study of approximately 649 matings and 60,000 individuals are summarized in the present paper. The solution of the problem is, however, barely begun, for these data, although they bring out many interesting and consistent facts, throw practically no light on the mechanism by which abnormal males rather than normal females are produced from fertilized eggs. Further studies in cytology and embryology must be undertaken if the problem is to be finally solved.

The technique involved in rearing and caring for the wasps is fully described in the Journal of Heredity for June 1921 (WHITING 1921 b). The work has been carried on at the STATE UNIVERSITY OF IOWA, Iowa City. My thanks are due to Professors BALDWIN, NUTTING and WYLIE, and to the members of their respective departments for help and kindly interest and the use of space and equipment. Professors FARR and WHITING have given helpful criticism and encouragement.

CROSSES PRODUCING PATROCLINOUS MALES

When black-eyed males are crossed with orange-eyed females, orange males and black females are expected by sex-linkoid inheritance. A few black males appear, however, and these must receive the factor for black from the male parent, since unmated orange females have never produced black-eyed offspring. From the reciprocal cross of black female by orange male all black offspring are expected, while patroclinous males in this case would be orange. In matings of this second type there were produced 3824 black males, 2536 black females and but 1 orange, a misshapen male which died as a pupa.

Crosses which have produced black-eyed patroclinous males may be divided into two classes, those made early in the work, before the derivation of definitely selected stocks, and those made between these various stocks after their establishment. Within each of the two classes are subdivisions, since two types of material are involved, one arising from a mated female caught in Lancaster, Pennsylvania, and the other from a mated female taken in Iowa City.

Early crosses

Lancaster material

Table 1 gives a summary of the results obtained from crossing the earlier orange-eyed female descendants of the orange-eyed mutant with related

black-eyed males of the Lancaster material. In this table, as well as in tables 2, 3 and 4, the matings are grouped into those which produced patrocliny and those which did not. It can be observed from table 1 that the results of the majority of crosses were contrary to expectation, since all the males produced were not like the mothers. The exceptional black-eyed males must have come from fertilized eggs, since virgin orange females have produced only orange-eyed offspring. The percentage of patrocliny,

$$\frac{\text{black } ♂♂ \times 100}{\text{total blacks}},$$

is here 8.09 ± 0.45, while the percentage of females,

$$\frac{♀♀ \times 100}{\text{total}},$$

is 39.18 ± 0.53.

It seemed possible that a high percentage of patrocliny might mean a correspondingly high percentage of abnormal fertilizations with perhaps much mortality of fertilized eggs and perhaps also a low percentage of females. Were this the case one might expect a negative correlation between percentage of patrocliny and percentage of females in each mating. No correlation was found to exist, however, between these percentages within this group or within any of the other groups to be discussed.

TABLE 1

Summary of early miscellaneous crosses.

TYPE OF MATING	NUMBER OF MATINGS	BLACK ♂♂	ORANGE ♂♂	BLACK ♀♀	BLACK ♂♂ ×100 / BLACKS	♀♀ ×100 / TOTAL
Lancaster	11	0	647	194		23.06 ± 0.98
miscellaneous	44	121	1551	1300	8.51 ± 0.47	43.74 ± 0.60
Total	55	121	2198	1494	8.09 ± 0.45	39.18 ± 0.53
Lancaster and Iowa City miscel-	14	0	589	598		50.37 ± 0.98
laneous	8	19	314	254	6.95 ± 0.96	43.27 ± 1.37
Total	22	19	903	852	2.18 ± 0.31	48.02 ± 0.79

Mixed Lancaster and Iowa City material

In September 1922 a stray female was taken in Iowa City, under circumstances which led us to suppose that she was not an escape from our

stocks. A study of her descendants confirmed this supposition for several striking differences were brought to light. She had already mated. Two of her male descendants were crossed to orange females of stock 5, Lancaster material. Their offspring total 71 orange males, 86 black females, and 0 black males, with a 0 percent of patrocliny and 54.77 ($\pm$2.63) percent of females. These numbers are too small to be significant in themselves, but results are consistent with later data.

Some of the progeny from the two matings just described were used in the derivation of stock 12. Crosses involved in this derivation, where black-eyed males were mated to orange-eyed females, are summarized in table 1. In many cases the females had mated with more than one brother, so these data are not always the results of single crosses. Percentage of patrocliny is here 2.18$\pm$0.31, of females 48.02$\pm$0.79.

These results indicate to some extent that percentage of patrocliny is higher in Lancaster material (8.09) than in a mixture of it with the Iowa City material (2.18) and that it is 0 percent in the progeny from crosses of Lancaster orange females by Iowa City black males. Percentage of females is highest in Iowa City matings (54.77), lower in the crosses of mixed material (48.02), and lowest in the Lancaster material (39.18). This would suggest a negative correlation between percentage of patrocliny and percentage of females, when the ratios derived from the totals of the various groups are considered. Numbers are too small to be more than suggestive up to this point, however.

Crosses of stocks

Many of the miscellaneous crosses just summarized were made by Doctor WHITING in breeding for other purposes. My work has been concerned with the results of crosses between various definitely selected stocks in an attempt to determine whether or not genetic differences existed which might throw some light on the causes or methods of production of patroclinous males.

Females were isolated as pupae and mated once to the selected male. For the most part the males were killed immediately after mating and only rarely was a single male used with two females. In such cases an interval of several hours passed between matings. Previous experiments have shown that a male may mate as many as thirteen times in immediate succession and still produce daughters, although in slightly decreasing percentages, and that after a five-hour rest, apparently normal fertility is restored. Results of mating two females to the same male a day or two apart are probably comparable as far as numbers of fertilized eggs are

concerned. Environmental conditions were kept as constant as possible throughout. Wasps were raised at 30° C and taken out of the incubator only for the purpose of counting. The derivation and character of the various stocks used in the following crosses are taken up in detail elsewhere (WHITING 1924). They will be briefly described below as they are considered.

TABLE 2

Summary of crosses between females of stocks 3, 5, 10 and 12 and stock-1 males.

TYPE OF MATING	NUMBER OF MATINGS	BLACK ♂♂	ORANGE ♀♀	BLACK ♀♀	BLACK ♂♂ ×100 / BLACKS	♀♀ × 100 / TOTAL
3 × 1	8	46	1084	371	11.03±1.03	24.71±0.74
5 × 1	16	0	552	297		34.98±1.10
	35	93	1368	929	9.09±0.60	39.24±0.66
Total	51	93	1920	1226	7.05±0.47	37.85±0.56
10 × 1	11	0	487	198		28.90±1.16
	44	153	2623	1793	7.86±0.38	39.15±0.48
Total	55	153	3110	1991	7.13±0.37	37.89±0.44
12 × 1	12	0	629	728		53.64±0.97
	7	35	350	296	10.57±1.07	43.46±1.21
Total	19	35	979	1024	3.30±0.35	50.24±0.74

Females of stocks 3, 5, 10 and 12 by stock-1 males

Stock 1 was derived from Lancaster material and has black eyes. Stocks 3, 5 and 10 were likewise derived from Lancaster material and have orange eyes. Stock 12 was derived from crosses of stock 5 by Iowa City material. In table 2 are summarized the results of these crosses. Crosses 5×1 and 10×1 are very similar in percentages of patrocliny and of females, while 3×1, probably less significant because of smaller numbers, is higher in percentage of patrocliny and lower in percentage of females. Cross 12×1, a cross between a stock involving both Lancaster and Iowa City material and a Lancaster stock, gives the lowest percentage of patrocliny and the highest percentage of females.

Females of stocks 3, 10 and 12 by stock-9b males

Stock 9b was derived from Lancaster material and has black eyes. Relatively few tests were made of it because of its close relationship to

stock 1. It was not tested with females of stock 5. The results of these crosses are summarized in table 3. Here again, where stock-12 females are involved, the percentage of patrocliny is lowest, that of females highest. The percentages in this table are of less significance than in table 2 since numbers are smaller.

TABLE 3

Summary of crosses between females of stocks 3, 10 and 12 and stock-9b males.

TYPE OF MATING	NUMBER OF MATINGS	BLACK ♂♂	ORANGE ♂♂	BLACK ♀♀	BLACK ♂♂ × 100 / BLACKS	♀♀ × 100 / TOTAL
3 × 9b	1	0	82	67		44.96±2.75
	11	29	636	483	5.66±0.64	42.07±0.94
Total	12	29	718	550	5.00±0.60	42.40±0.87
10 × 9b	5	0	208	71		25.44±1.18
	7	11	232	155	6.62±1.21	38.94±1.61
Total	12	11	440	226	4.64±0.80	33.38±1.21
12 × 9b	6	0	240	350		59.32±1.36
	3	12	142	173	6.48±1.14	52.90±1.82
Total	9	12	382	523	2.24±0.40	57.03±1.07

Females of stocks 3, 5, 10 and 12 by stock-11 males

Stock 11, unlike the black-eyed stocks 1 and 9b, was derived from Iowa City material. It is therefore unrelated to the orange females of stocks

TABLE 4

Summary of crosses between stock-11 males and females of stocks 3, 5, 10 and 12.

TYPE OF MATING	NUMBER OF MATINGS	BLACK ♂♂	ORANGE ♂♂	BLACK ♀♀	BLACK ♂♂ × 100 / BLACKS	♀♀ × 100 / TOTAL
3 × 11	25	0	1465	2203		60.05±0.53
5 × 11	20	0	1462	1446		49.72±0.53
10 × 11	34	0	976	1674		63.16±0.62
12 × 11	6	0	290	387		57.16±1.28
	5	16	281	163	8.93±1.36	35.43±1.49
Total	11	16	571	550	2.82±0.39	48.37±0.99

3, 5 and 10, but is related to those of stock 12. Striking results were obtained (table 4) when it was crossed to the unrelated orange females, for no patroclinous males were produced in the 79 matings which resulted in 3903 orange males and 5323 black females. Orange-eyed females of the related stock 12 by stock-11 males produced patrocliny, although only eleven matings of this type were made. As in crosses involving

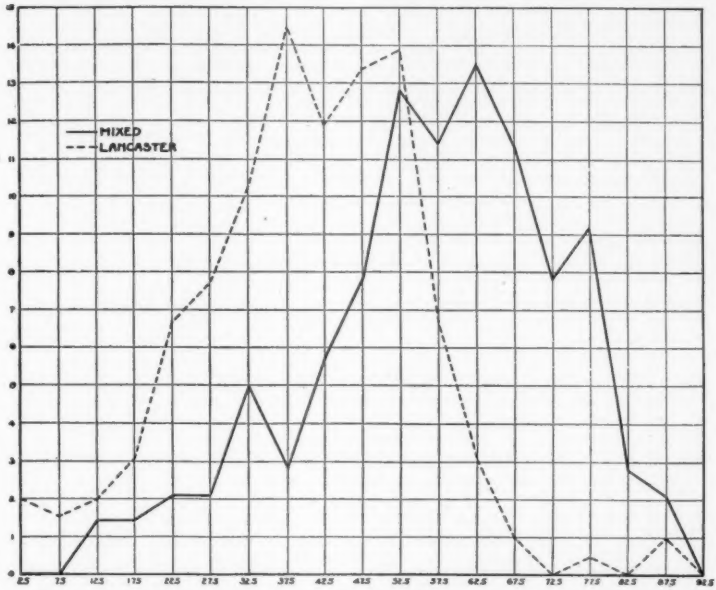


DIAGRAM 1.—Percentage frequencies (ordinates) of percentages of females (abscissae) for the two groups, Lancaster, and Lancaster and Iowa City mixed.

stock-12 females and males of stocks 1 and 9b, the percentage of patrocliny is low, the percentage of females high.

Summary of crosses producing patroclinous males

The percentages of females to total offspring and of black males to total blacks for each mating have been grouped into those involving only Lancaster material and those involving both Lancaster and Iowa City materials. Diagram 1 shows percentage frequencies (ordinates) of percentages of females (abscissae) for the two groups. Diagram 2 shows percentage frequencies (ordinates) of percentages of patrocliny (abscissae). It is evident from these diagrams that the higher percentages

of females and the lower percentages of patrocliny on the whole, are present where Iowa City stocks are involved. The arithmetical means of the percentages of females (diagram 1) are 54.3 for the mixed group and 37.59 for the Lancaster group. The arithmetical means of the percentages of patrocliny (diagram 2) are 1.32 for the mixed group and 6.40 for the Lancaster material.

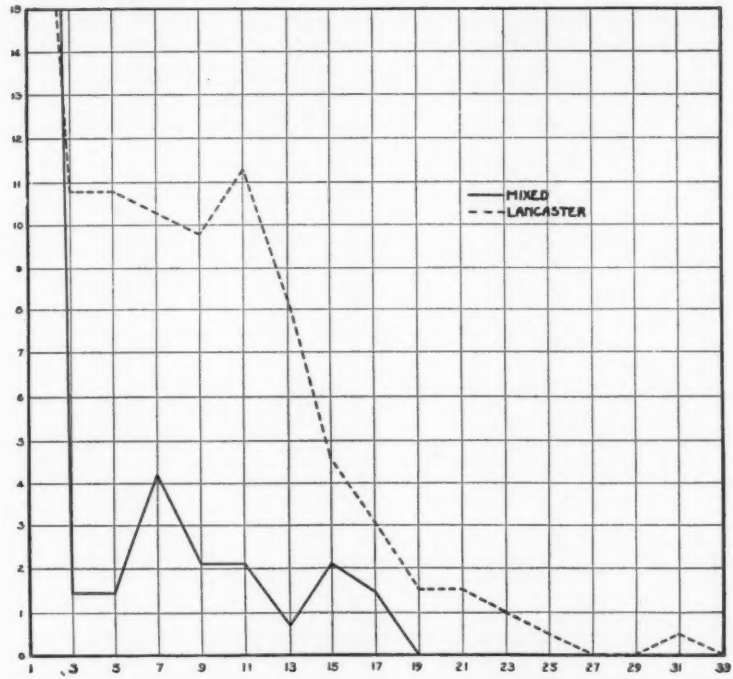


DIAGRAM 2.—Percentage frequencies (ordinates) of percentages of patrocliny (abscissae) for the two groups, Lancaster, and Lancaster and Iowa City mixed. Percentage frequencies centering about 1 percent of patrocliny are 84 for the mixed group, and 25.3 for the Lancaster group.

As mentioned above, the percentage of patrocliny and of females for each mating within each type of cross showed no correlation. When these percentages of all the matings in all of the types of crosses were considered together, the coefficient of correlation was found to be -0.323 ± 0.034. The coefficient of correlation between the percentages of females and of patrocliny of the sums of offspring in each type of mating was found to be -0.86 ± 0.04. A comparatively high percentage of patrocliny in one

type of cross means, then, a comparatively low percentage of females in the same type. This would seem to indicate that the genetic differences which determine these percentages are isolated in the stocks in such a way as to give negative correlation in crossing.

With the exception of the crosses of females of stocks 3, 5 and 10 by stock-11 males, where no patrocliny is produced, crosses involving stock-12 females produce the lowest percentages of patrocliny and the highest percentages of females. A study of tables 1 to 4 shows that this is due to the fact that in a high percentage of the matings no patrocliny is produced, rather than to the fact that lower percentages are produced in a larger number of matings. In other words, in matings involving stock-12 females, where patrocliny is produced it is in at least as high a percentage as in other types of crosses, but it is produced in fewer matings of this type.

Another point brought out by the study of these tables is that crosses of any stock with a related stock produces a lower percentage of females and a higher percentage of patrocliny than crosses of that stock with an unrelated stock.

PATROCLINOUS MALES

Abnormalities in external anatomy

Since males are normally produced from unfertilized eggs, and since fertilized eggs ordinarily produce females, it might be expected that patroclinous males, arising as they do from an abnormal or at least uncommon process, would be abnormal morphologically and physiologically. Morphological abnormalities are of fairly common occurrence in the regular stocks, among males and females produced in the orthodox manner. Tests of such abnormal individuals or freaks have shown them to be fully fertile in most cases, when they produce normal offspring. Freaks are likewise found among the patroclinous males but in a larger percentage. When the total offspring of all the crosses between the stocks are grouped according to normal and abnormal gross morphology we find that 3.54 ($\pm$0.57) percent of patroclinous males, 0.16 ($\pm$0.02) percent of orange males, and 0.61 ($\pm$0.05) percent of black females are freaks. The differences between these percentages are all more than four times their probable errors and therefore doubtlessly significant. It is found in summarizing the results within stocks that the females include a higher percentage of freaks than do the males. The higher percentage found in females when stocks are crossed is, therefore, probably not due to the fact that the females are here heterozygous.

Defects in antennae, abdominal sclerites, and thorax, abnormal leg number, incomplete digestive tract, abnormal genitalia, wings and eyes were all found among the females studied, the abnormal abdomen in highest percentage. With the exception of antennal defects these were duplicated in the orange males, but in lower percentages, while in the patroclinous males the defects were divided among abnormal antennae, thorax and legs, and digestive tract.

Occasionally pupae die within the cocoons and are dissected out and studied when counts are being made. Larvae also pupate at times without spinning cocoons and either metamorphose normally before counts are made or are shaken into the etherizing bottle as pupae. Ordinarily these are studied and recorded with dead pupae under the general head of "pupae counted." This includes two groups, then, pupae which were in cocoons and unable to emerge, and naked pupae which might or might not have developed if left for a longer time. Whenever a naked black-eyed male pupa was shaken into the etherizing bottle it was returned to a fresh vial, labelled "np" and allowed to develop if possible. If it produced an adult a note was made to the effect that it had been a naked pupa. The heading "pupae counted" here includes, as in the other two groups, pupae unable to emerge, naked pupae which could not develop, and those which could. In summarizing it was found that 8.60 ($\pm$0.87) percent of patroclinous males fall in this group, 0.77 ($\pm$0.05) percent of orange males, and 0.72 ($\pm$0.05) percent of black females. The differences between 8.60 percent and the other two percentages are significant, but that between 0.77 percent and 0.72 percent is not. Of patroclinous males produced by cross of stock 3 by 1, 32.6 ($\pm$4.58) percent were naked pupae, as were 20.6 ($\pm$4.78) percent of those produced by stock 3 by 9b, so that stock 3 seems to have a special tendency in this direction.

Tests of fertility

In addition to the fact that anomalous males tend, more than other individuals, to be abnormal in morphology, they show a striking tendency to sterility. When fertile they are usually but slightly so and except for a few early cases breed like blacks, indicating that gonads as well as eyes have come from paternal nuclei. P. W. WHITING, after preliminary tests of the patroclinous males, states (WHITING 1921) that six black males produced orange daughters only, showing that although their eyes were paternal in origin their gonads were from maternal nuclei. They were therefore mosaics. Six black males produced black daughters only and hence their gonads as well as their eyes were of paternal tissue.

The six patroclinous males breeding as orange were mated to orange females. One was crossed to five females, each of which produced daughters. Two others were mated twice and were fertile in but one mating each. Five matings only were observed and in the other seven cases the individuals were merely left together for a time. Percentage of females is here 26.44 ± 0.83, which compares well with those of normal crosses. Six males were found by tests to occur in the second class, that is, they bred as blacks. Ten matings involving these six males were made, seven of which were observed. One male was mated four times and produced daughters in but one mating. Another was mated twice and proved fertile in each. The female percentage is in this case but 3.60 ± 0.39 although including one mating with a fair percentage of females. The difference between these two percentages is twenty-four times its probable error and therefore doubtlessly significant. We have here, then, an indication that male gonads containing paternal elements may be of low fertility, a possibility which further work strengthens.

Subsequent tests have revealed no more black-eyed patroclinous males breeding as orange, so that our data on them is meager. The tests here recorded suggest that male gonads derived from maternal tissue function normally although present in an organism part of which is of paternal origin.

The above summary includes only 30 percent of the black-eyed males tested, however, for 70 (± 4.88) percent were found to produce no offspring. Fifty-three matings involving twenty-eight of these anomalous males of early Lancaster miscellaneous stock produced no daughters, although their mates produced, in all, 3211 sons. Matings were observed in seventeen cases and male offspring ranged from 1 to 290 per male. Ten males were mated twice each, two males five times each, one male four times, and two males three times each, no mating resulting in the production of daughters.

In making these tests of patroclinous males it is, of course, especially important to have the females virgin, and essential that they be orange, if the nature of the male gonads is to be ascertained. The method of making matings used in the later tests differs somewhat from that followed in previous crosses. Since so large a percentage of anomalous males was found to be sterile it was felt that greater opportunity for mating might increase the chances for fertilization by any scattered spermatozoa which might be present. Therefore, after the first few tests a mating of each pair was observed and they were then left together for several hours, usually about twenty-four. They were often seen to mate numerous times

during this period. The male was then isolated, fed honey water, and kept in a cool place, while the female was given caterpillars and set in an incubator at 30° C. If a male was mated to more than one female an interval was allowed to elapse between the matings. If a cross had produced interesting results the male involved was tested again with one or more other females.

Tests of patroclinous males resulting from the early Lancaster miscellaneous crosses have already been discussed. Seventeen patroclinous males from the mixed miscellaneous series were tested. Nineteen matings, all but four observed, involving fourteen of these males, produced 2242 sons and no daughters. The number of sons per male tested ranged from 50 to 459. Five of these males were mated, each to two females, resulting in no daughters in either case. One male was mated to five females and produced no daughters in four of the matings, and only two in the fifth,—two females to a total of 584 males. Two males, mated to one female each, produced a few daughters. Summarizing, we have males tested 17, males sterile 14, males partially fertile 3. The three fertile males produced 11 daughters among 767 offspring, or 1.43 ($\pm$0.23) percent. The 11 daughters of these anomalous males were all black-eyed, although the mothers were of orange-eyed stock 5. In further tests these apparently sterile males might have sired a few daughters, but this evidence shows conclusively that the majority of patroclinous males, 82.35 ($\pm$6.27) percent, in this case, are practically sterile. This conclusion is strengthened by further tests.

Four patroclinous males from a cross of stock 3 by 1 were tested. Three sired no daughters although their mates produced 220 orange males. One male proved less sterile, since he produced 1 black daughter among 30 offspring. This gives a percentage of sterility of 75.00 $\pm$ 14.56, a female percentage of 3.33 $\pm$ 2.09.

Thirty-two matings of anomalous males from stock 5 by 1, involving twenty-four males, gave negative results, although the females produced, in all, 3754 males, with a range of sons per male tested, from 10 to 416. Matings were observed in twenty-seven of these cases, but individuals were not left together afterwards. Eight males were mated, each to two females, and three other males were given two females each. In this group only three males gave daughters, 7 black-eyed females to 365 males, or a percentage of females of 1.88 $\pm$ 0.34. Two of these matings were observed. Summarizing, we find that males tested were 27, males sterile 24, males slightly fertile 3, a percentage of sterility of 88.88 $\pm$ 4.20.

A larger number of the black-eyed males from crosses of stock 10 by 1 were tested, than of those of the other groups. Fifty-eight matings involving fifty males resulted in no daughters and 6606 sons, ranging in number from 8 to 433 per anomalous male tested. Twelve of these matings were observed, but the individuals were not left together. Thirty-nine were observed and left together for twenty-four hours and no record was made of the other seven. Six males were tested twice with negative results. One male was tested twice and proved fertile in one mating; one male was tested three times, two tests proving fertile; and still another was tested four times with two tests fertile. Here again we have indications that increased numbers of tests of individuals might show that they have a few viable spermatozoa. Eighteen matings involving twelve males produced 36 black-eyed daughters to 2192 sons, a percentage of females of 1.61 ± 0.14. In all, sixty-two males were tested, fifty found to be sterile and twelve fertile, 80.64 (± 3.41) percent of sterility. In the latter group of eighteen, thirteen matings were observed and left twenty-four hours, one observed but the individuals not left together, and no record made of the others.

Six patroclinous males from crosses of stock 12 by 1, each mated to one female, gave no daughters, their mates having 368 sons, with a range of from 2 to 158 sons per male tested. Two males, each with one mating, produced 3 daughters to 311 sons, a female percentage of 0.95 ± 0.36. Tests of eight males show that 6, or 75.00 (± 10.30) percent, are sterile. The number of tests is here too small to be of much significance.

Daughters failed to result from eleven tests involving ten males from crosses of stock 3 by 9b. Sons produced were 1215, with a range of from 20 to 202 sons per anomalous male. One male produced in one test 5 daughters to 109 males, a female percentage of 4.38 ± 3.91. This gives 90.90 (± 6.09) percent sterility, of little significance because of small numbers.

Three patroclinous males, one tested twice, from crosses of stock 10 by 9b, gave no daughters, although their mates produced 190 sons, with a range of from 16 to 87 per male tested. No fertile anomalous males were tested in this group.

Two matings of one male from crosses of stock 12 by 9b gave 136 sons and no daughters. One male in a single mating gave 1 daughter to 25 sons. Percentage of females is 3.84 ± 2.25, of sterility 50.00 ± 23.74.

No anomalous males were produced by the crosses of stocks 3, 5 and 10 by 11.

Eight males from crosses of 12 by 11 were tested. Eight matings involving six males were observed. 750 males were produced by their

mates, ranging from 11 to 202 per male, but no female offspring appeared. Two other black-eyed males, when crossed with orange females, proved to be slightly fertile, since the crosses resulted in 297 orange males, 10 black females, and what was probably 1 black anomalous male, the only case where anomalous has been produced by anomalous. Percentage of females is 3.24 ± 0.65. Unfortunately, no test was made of the black, presumably anomalous male, produced by an anomalous. Six males out of eight tested, or 75.00 (± 10.30) percent, were sterile.

On table 5 are tabulated the percentages of sterile anomalous males and the percentages of females produced in crosses of the fertile anomalous males. In some cases numbers are too small to be significant, but

TABLE 5

Summary of patroclinous males.

SOURCE	TESTED PATROCLINOUS MALES			PROGENY FROM MATINGS OF FERTILE PATROCLINOUS MALES		
	Sterile	Total	Percent sterile	♀♀	Total	Percent ♀♀
Lancaster miscellaneous	28	40	70.00±4.88	30	833	3.60±0.83
				329	1244	26.44±0.83
Lancaster and Iowa City miscellaneous	14	17	82.35±6.27	11	767	1.43±0.23
3 × 1	3	4	75.00±14.56	1	30	3.33±2.09
5 × 1	24	27	88.88± 4.20	7	372	1.88±0.34
10 × 1	50	62	80.64± 3.41	36	2228	1.61±0.14
12 × 1	6	8	75.00±10.30	3	314	.95±0.36
3 × 9b	10	11	90.90± 6.09	5	114	4.38±3.91
10 × 9b	3	3	100.00			
12 × 9b	1	2	50.00±23.74	1	26	3.84±2.25
12 × 11	6	8	75.00±10.30	10	308	3.24±0.65
Total	145	182	79.67± 2.03	433	6236	
Total fraternities containing black				104	3873	2.68±0.14

it is apparent at a glance that percentage of sterility is high in patroclinous males produced by all types of crosses, and that when these males are fertile they are but slightly so, with the single exception of the six mosaics breeding like orange. The percentage of sterile patroclinous males from the cross of 10 by 1 was used as a basis of comparison, since it is most significant, and the probable errors of the differences between it and the other significant percentages found. From this it is evident

that very few or no significant differences (less than four times their probable errors) exist between it and percentages of sterile patroclinous males from Lancaster miscellaneous, Lancaster and Iowa City mixed, 5×1, 12×1, or 12×11. It has been already observed that the difference between percentage of females produced by patroclinous males breeding like orange, and that produced by those of the same strain breeding like black, is strikingly significant, about twenty-four times its probable error. The percentage of females produced by patroclinous males from 10×1 was likewise used as a basis of comparison. The difference between it and the percentage of females produced by the six patroclinous males breeding like orange was found to be about forty times its probable error. The differences between it and the percentage of Lancaster miscellaneous, Lancaster and Iowa City mixed, 5×1, 12×1, and 12×11 are here again less than four times their probable errors and therefore of little or no significance. It is quite possible that many tests of each black male might move many of these so-called sterile males into the class of slightly fertile, but the results would still be strikingly different from the normal condition, where most males are fully fertile at the first test. Omitting the six mosaic males breeding like orange, we find that altogether 104 black females were produced among 3873 individuals, a female percentage of 2.68 ± 0.14.

The chance that orange-eyed brothers of anomalous males might be mosaics with paternal gonads was early thought of and one such case, mentioned above, was found. The task of testing orange-eyed brothers would have been endless, for there are no external signs whereby such males could be singled out. Whenever these males have been used in crosses, however, they have bred normally, with the exception mentioned above. The fact that percentages of abnormalities and of pupae are so low in the class of orange males might give a slight indication that if these mosaics do occur they are rare.

Patrocliny was discovered because of the difference in eye color and this same difference enabled us to learn of the mosaic character of some of the males. Other character differences would throw still more light on the subject of the make-up of the patroclinous males, especially a constant thoracic character. There have been no thoroughly satisfactory means of determining the source of the tissue making up the thorax, however. Defects in the fourth branch of the radius vein occur in several of the stocks. These are hereditary, but are very variable and much affected by environmental conditions, which facts lessen the value of this character. Stock 1 at 30° C produces a defective individual very rarely,

so that it can in general be described as normal as far as this character is concerned. Stock 10, on the other hand, produces a high percentage (90) of defectives under the same environmental conditions. A study of this character in the offspring of 10 by 1 gives interesting results. The orange males of this cross are haploid for defective, the black females heterozygous, containing factors for both normality (incompletely dominant) and defect, while the constitution of the thorax of the anomalous males is unknown. Being part of a male individual it is presumably haploid and if defective is probably maternal, although there is a very slight chance of its being paternal. In summarizing the individuals produced by the fifty-five crosses of 10 by 1 it was found that 2815 out of a total of 3082 orange males, or 90.85 ($\pm$0.36) percent, were defective. Among the black heterozygous females 101 out of a total of 1956, or 5.16 ($\pm$0.33) percent, were defective. These numbers represent the extent to which normal is not dominant over defective, and are sufficiently large to make the difference between the percentages significant. Fifteen defective anomalous males were produced in a total of 150, or 10.00 ($\pm$1.64) percent. The difference between this percentage and that of defective among females is but 2.88 times its probable error and therefore not significant. If chances for thorax to be maternal or paternal were even, there ought to be, on the basis of the ratio obtained for the orange males, 82.5 normal individuals and 67.5 defective. The low percentage of defectives argues for either a paternal origin of the thorax in most cases or for a heterozygous diploid condition in some cases. If the thoraces are of either maternal or paternal origin, the fifteen anomalous males with defective wings are of maternal origin and represent 90 percent of the genetically defective, for the orange males show that amount of defect. The other 10 percent, or 1.66, of the genetically defective appear normal, while 133.33 are both genetically and somatically normal. This would mean that approximately 133.33 of every 150 thoraces, or 88 percent, are of paternal origin, while 16.66 of every 150, or 22 percent, are of maternal origin. Gonads are haploid and paternal in the great majority of cases, the head is paternal or heterozygous, but probably the former, since a diploid head would be expected to have the antennae and brain-complex of a female. The thorax is apparently in some cases maternal, but in the majority of cases paternal, although there is the possibility of its being heterozygous or mosaic.

Defects in the fourth branch of the radius, r_4, are separated into eight grades (WHITING 1923), the highest grade (8) denoting complete absence of the vein from both wings, the lowest grade (1) denoting a very slight

break in the vein of one wing. Grade 4 denotes either the complete absence of the vein from one wing or its partial reduction in both. The mean grade of defect was found for the defective wasps in each group of the offspring of 10 by 1. In the orange males it is 5.46, in the black females it is 2.34, and in the black patroclinous males it is 2.92.

If both wings of each patroclinous male having defective venation were of maternal origin, we would expect the mean grade of defect for them to be the same as for the orange males. If the defective wings were heterozygous, we might expect a grade of defect similar to that of the heterozygous females. We have such a similarity in grade here, but it would seem more likely from what we know of the constitution of these males that the thorax may at times be mosaic, so that one wing is defective, being maternal, while the other, paternal, side is normal. This would of course lower the mean grade of defect. A record kept of the amount of defect in each individual wing would probably not help greatly in deciding this question, for the character is so extremely variable.

Stock 3 is also an orange defective stock producing a somewhat higher percentage of defectives than stock 10. In crosses of 3 by 1 it was found that orange males were defective in 995 cases out of 1066, or in 93.33 ($\pm$0.52) percent. In black females there were 22 defectives among 364 individuals, or 6.04 ($\pm$0.83) percent. Among the patroclinous males 8 out of 44, or 18.18 ($\pm$3.89) percent, were defective. The difference between orange males and each of the other groups is significant, but the difference between percentage of defect in black females and patroclinous males is less than four times its probable error. Here, again, the patroclinous males are much less defective than the orange males, and somewhat more so than the heterozygous females.

A recessive factor which causes the ventral surface of the thorax to be very black when the individuals are raised at 30° C has been discovered in stocks 11 and 12. When present in double dose in stock-11 females the "sooty" condition does not show, but in stock 12 both sexes show the character strongly. When 9b and 1 males were crossed to stock-12 females the daughters were light, the orange-eyed males sooty, since their mothers were homozygous for this factor, while the black-eyed males in all cases, although slightly darker than the females, were very much lighter than the orange males, indicating that in these crosses patroclinous males have their thoraces formed from paternal tissue.

Internal anatomy

The abdomens of twenty-seven patroclinous males were fixed in Carnoy's solution, imbedded, sectioned, and stained with triple stain. The slides were studied in order to learn whether or not sterility were due to morphological malformations. Twenty-three of these were found to be normal as far as gross morphology was concerned. Both testes were present and connected completely with the sperm sacs which were normal in appearance, extending posteriorly to form a normal penis. Of these twenty-three, eleven had been tested by mating to virgin females, while twelve, one of which had five legs, and another a defective wing, had been fixed without previous tests. Of the eleven tested, seven were sterile, although four were tested twice and one had been mated three times. The remaining four had produced, respectively, three daughters in four matings, eight daughters in two matings, two daughters in three matings, and two daughters in one mating.

One male which was studied and found normal internally had refused to mate when attempts had been made to test him. He had one defective leg. In another male, which had been tested twice and found sterile, complete connections could not be made out between testes and sperm sacs, but a few sections in this region had been lost, so connections may have been normal. Freak 44, a male with abnormal external genitalia, had been set with a female, and was left with her about twenty-four hours, during which time he attempted to mate. She produced no daughters and it was found upon sectioning him that although both testes were present, one only was connected with a single sperm sac and that this ended blindly instead of being elongated to form the penis. Another male, freak 69, was found to be quite abnormal. Both testes were present, but one sperm sac was absent entirely. Connection was made with the remaining one and its corresponding testis and the single sac elongated to form a penis. Tests with two females resulted in no offspring. Another male, freak 73, appeared quite normal except that the sperm sacs were separated somewhat, anteriorly, instead of lying close together throughout their entire length, as is the usual condition. This male was tested twice and found sterile. Summarizing: Of the twenty-seven tested, four had external abnormalities, while four, or possibly three, were abnormal internally, and in one case only were there both external and internal abnormalities. All abnormal individuals tested were sterile. Of the twenty-three normal internally, eleven were tested, seven of which proved sterile.

DAUGHTERS OF PATROCLINOUS MALES

The daughters of patroclinous males fall into two groups according to eye color. The orange-eyed group numbers 329, the offspring of six males, the black-eyed group numbers 104, the offspring of thirty males. This re-emphasizes the point already made that the patroclinous black-eyed males with orange-bearing gonads are more fertile than black-eyed males with black-bearing gonads, since the former average 54.8 daughters each, the latter 3.46 daughters each.

Abnormalities in external anatomy

Among the offspring of the matings of patroclinous males breeding like orange there are recorded no freaks among 724 males, and four freaks among 329 females, or 1.21 ($\pm$0.36) percent. Two of these had abnormal abdomen and two abnormal genitalia. The results of matings of the blacks which produce patroclinous males are quite different. Among the 3766 males produced, only 8, or 0.212 ($\pm$0.04) percent, were freaks. Among the 104 daughters there were 32, or 30.73 ($\pm$3.04) percent, freaks. The difference between percentages of females in the two groups is 9.64 times its probable error and therefore certainly significant. Of the eight male freaks five had defective digestive tracts, one abnormal genitalia, one both of these abnormalities, and one a small wing.

The numerous freaks found among the black-eyed daughters were unusual in the extent of abnormality present. Eleven of the thirty-two had abnormal abdominal sclerites of several types. A few of these are illustrated in figures A, D, G, H, I, J and K, plate 1; eight had thoraces much deformed with one or more legs deformed or missing. Figures L and M show this. In figure L (freak 41) five legs were normal, the right mesothoracic leg ended in a blunt stump, while an additional jointed appendage grew from the ventral mid-line of the thorax at the base of the left mesothoracic leg. A tumor-like outgrowth was also present on the right ventral surface. As shown in figure M (freak 53) the left mesothoracic leg is entirely absent while there is a large tumor-like growth beneath the left wings. The thorax is generally deformed and twisted. Each of the following classes contained one individual: defective digestive tract, an antenna missing, no external genitalia, abnormal abdomen and defective genitalia, shrivelled wing, abnormal abdomen in addition to shrivelled wing, abnormal thorax with right primary wing missing, defective tarsi on right third leg, shrivelled wings with a small undeveloped leg and abnormal abdomen, and wings crumpled.

Explanation of Plate 1

All figures have been drawn with camera-lucida. Drawings have been reduced three-fifths. Final magnifications are ×20, except in figure C which is magnified 40 diameters.

Figures A to M.—Daughters of patroclinous males.

Figures A to C.—Freak 13.

Figure A.—Dorsal view of abdomen showing abnormal sclerites, extra bristle and gonapophysis.

Figure B.—Lateral view of posterior end of abdomen showing two extra gonapophyses and two bristles not normally present.

Figure C.—Head with left eye much reduced.

Figures D to G.—Freak 2, sister of freak 13.

Figure D.—Ventral view of abdomen showing asymmetry in posterior sclerites.

Figure E.—Right side of thorax. Prothoracic and metathoracic legs normal. Mesothoracic leg not visible from this angle. Wings normal.

Figure F.—Left side of thorax. Right mesothoracic leg just anterior to left metathoracic leg; much reduced left mesothoracic leg pushed dorsally and arising from edge of abnormal circular region of thin chitin. Wings unexpanded on this side.

Figure G.—Dorsal view of abdomen, sclerites abnormal.

Figures H to K.—Dorsal views of abdomens of four females showing abnormal arrangement of sclerites.

Figure H.—Freak 4. Note immature genitalia. Additional defects not shown in drawing are unexpanded wings, flattened and twisted leg, deformed thorax, attachment to abdomen of shreds of larval skin, and head drawn back against thorax.

Figure I.—Freak 20. Defects not shown in drawing are reduction of left mesothoracic leg to a stump, and presence of two small tumors on thorax.

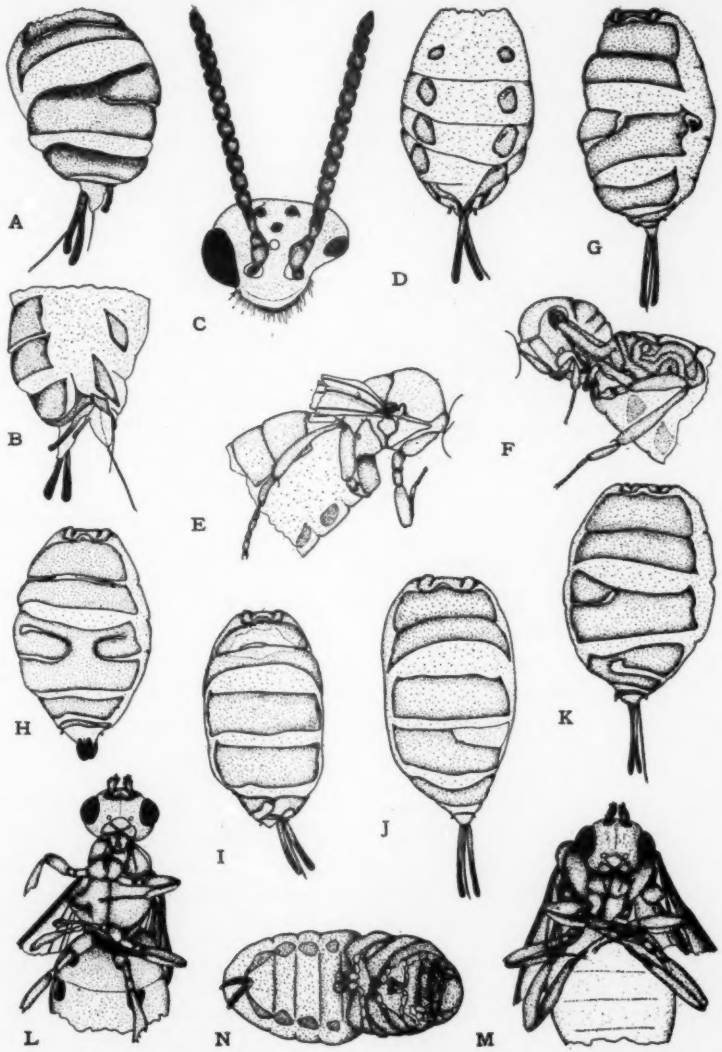
Figure J.—Freak 24.

Figure K.—Freak 6, sister of freaks 13 and 2.

Figure L.—Freak 41. Right mesothoracic leg undeveloped beyond femur. Extra jointed appendage at base of left mesothoracic leg. Two tumor-like projections on right ventral part of mesothorax.

Figure M.—Freak 53. Left mesothoracic leg absent. Large tumor on left side beneath wings. Thorax generally deformed.

Figure N.—Freak 56. Granddaughter of patroclinous male, lethal pupa. Larval covering abnormally persistent over head and thorax. Dorsal abdominal sclerites abnormal.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Freak 4 (abdomen shown in figure H) was one of the more striking cases of abnormality. She was unable to emerge from the cocoon and was dissected out. The genitalia were immature, the wings unexpanded, one leg flattened and twisted, the thorax deformed, shreds of larval skin still attached to abdomen, and head drawn back against the thorax. The pupal conditions had persisted beyond the time for eclosion. She moved about somewhat but was killed almost at once for study.

Freak 13 is shown in figures A, B and C. She was one of five sisters, one only of which appeared normal. Her left eye was much reduced in size and the dorsal sclerites and posterior part of her abdomen quite unusual. Two small extra gonapophyses were present as well as two extra bristle-like appendages.

Freak 2, sister to freak 13, is shown in figures D, E, F and G. Posterior abdominal sclerites were asymmetrical, dorsal sclerites were abnormal, left wings unexpanded, and both mesothoracic legs reduced in size and useless, the right one being held flat against the thorax and pointed directly to the left. The very abnormal left mesothoracic leg arises at the edge of a white circular patch which seems to consist of extremely thin integument not strongly chitinous. This female was also unable to emerge from the cocoon and had to be dissected out.

Freaks among the patroclinous males fell into three groups, antennal defects 1.51 ($\pm$0.33) percent, thorax and leg defects 0.50 ($\pm$0.23) percent, and digestive-tract defects 1.51 ($\pm$0.33) percent. Among their daughters there is one with missing antenna, 0.96 ($\pm$0.64) percent; eight with abnormal thoraces and legs, 7.69 ($\pm$1.68) percent; and one with defective digestive tract, 0.96 ($\pm$0.64) percent. The balance of abnormalities, twenty-two individuals, consisted of various combinations of these defects and the addition of others. Several of these daughters of anomalous were much more abnormal than have been any individuals found in the regular stocks, and this difference is further enlarged by breeding tests. Numbers of daughters of patroclinous males are too few in most groups for significant comparison of numbers of freaks within the groups. Percentages of freaks in groups large enough to be significant are found to be: for the orange group of Lancaster miscellaneous, 1.22 ($\pm$0.37) percent; for the black group, 10.00 ($\pm$1.16) percent; for the Lancaster and Iowa City miscellaneous, 54.54 ($\pm$10.11) percent; and for 10$\times$1, 47.22 ($\pm$5.60) percent.

Pupae among the offspring of matings of patroclinous males with orange-bearing gonads number 13 in 724 orange males, 1.65 ($\pm$0.24) percent, and 1 in 329 orange females, 0.30 ($\pm$0.20) percent. Among the

offspring of black-eyed males with black-bearing gonads, they number 23 in 3767 orange males, or 0.61 (±0.08) percent and 8 in 104 black females or 7.60 (±1.68) percent. Among the anomalous males they numbered 34 in 361 or 8.60 (±0.87) percent. The percentages are significantly higher in these two groups than in those produced under normal conditions.

Tests of fertility

It was not suspected at first that there might be anything unusual in the behavior of the daughters of patroclinous males in breeding tests, so that the orange daughters were tested only incidentally for other purposes, while the black-eyed daughters were not tested, since heterozygous females are so often not useful for tests involved. Altogether seventeen of the orange females were bred out and in each case results were as expected and fertility normal. 1449 males were produced and 208 females. In one case where an orange-eyed daughter of an anomalous male had been mated to a black-eyed male, she produced 5 black-eyed males, these anomalous males being, thus, grandsons of an anomalous male. They were normal in appearance. One was tested four times and found sterile, two were tested twice each with negative results, and a fourth was tested once, but inadequately, for the female with which it was placed produced only 3 orange sons. The fifth was not tested.

Of the 104 black-eyed daughters of anomalous males produced, the first thirty were not tested, since they were not needed in other crosses. Sixty-three of the seventy-four produced by the crosses of definite stocks were carefully tested. If the females were feeble, and many of them were, they were fed honey and water and caterpillars already stung by other females so as to prevent the spinning of webs that might entangle them. The cocoons containing these black-eyed females were watched from day to day and if they seemed unable to emerge the cocoon was carefully opened with a needle so that they could crawl out. In this way tests were made of many individuals which, had they been left to care for themselves, would have died. The eleven wasps not tested, either died as pupae or were too weak to notice food or caterpillars. Of the sixty-three tested, twenty-five were freaks and thirty-eight normal. Of the thirty-eight normal wasps, three were found dead while still well-fed and healthy-looking. They had laid no eggs although exposed to caterpillars for several days. Twenty-six laid eggs in varying numbers none of which developed. The eggs were often normal in appearance when laid but would shrivel in a day or two. Others seemed to be shrivelled when laid.

Two wasps, normal in appearance, laid no eggs, although they lived for some time, fed, and appeared healthy. Six others were tested, but no record was kept as to whether or not they laid eggs. One wasp laid a large number of eggs, six of which developed into larvae. Five of these died very soon although carefully cared for, while the sixth continued development until it pupated within a cocoon. When time for hatching, it was dissected out and found to have still an immature larval head and thorax. It was kept for two days during which time its spasmodic movements became weaker. It was therefore fixed and drawn (figure N). The mother had probably mated with an orange brother, for this individual was a female and apparently had orange eyes.

Among the twenty-five freaks tested, fifteen laid sterile eggs in varying numbers, six were sterile but no record was kept of egg numbers, while two laid no eggs although kept for some time. One only of the one hundred and four females produced an individual that matured normally. She had abnormal abdomen and was one of eight sisters, three only of which were normal in appearance. The single black-eyed male which she produced was mated to three virgin orange-eyed females and these produced, respectively, 89, 123 and 192 orange males, totalling 404 sons but no daughters. Complete sterility in the daughters of anomalous males occurred, therefore, in sixty-one out of sixty-three tested, or in 96.83 (± 1.49) percent. The two exceptions to this might almost be considered sterile also, since one produced no living adults and the other a single sterile individual.

Internal anatomy

The abdomens of thirty-five daughters of patroclinous males were fixed in Carnoy, sectioned, stained and studied. All but two were found to have eggs present in varying numbers and stages of development. Of these two, one was a pupa, the other, when tested, had laid no eggs. Four had very few eggs, while in two they were immature, since the individuals were in the pupal stage when fixed. In twenty-seven the eggs varied from a few to several, depending somewhat, perhaps, on the age of the female when fixed. Nurse cells corresponded in their presence and development, with the eggs. The poison sac and glands were normal in all, as was the uterus. The seminal receptacle was found in all except two, but it may possibly have been present in these originally, for the individuals had been almost dead when fixed and the tissues were not in good condition. Twenty-two of the sacs were filled with sperm, while eleven were empty.

The relation of the poison sac to the gut was observed incidentally in each case and found to be located to the left in nineteen cases, and to the right in sixteen cases. The digestive tract was complete in all but one individual where the mid-gut ended and the hind-gut began in blind sacs, and here the Malpighian tubules were absent also. This wasp lived eleven days, stung caterpillars, and laid four sterile eggs. Two of the females studied had not been tested, five laid no eggs, while twenty-three laid eggs that were not fertile. Three were pupae and of course had not been tested. The individual which produced the single black-eyed male seemed normal, but her seminal receptacle could not be found. She had been kept alive as long as possible in an attempt to secure more offspring, so that she was fixed when almost dead and the receptacle which in the best slides can be identified in but one or two sections might easily have been lost in this case. Four of the individuals studied did not sting caterpillars and of these one was found dead after three days in apparently good condition, another had a split sting, and the others, although left for ten and six days, respectively, with caterpillars, ignored them entirely.

From this it can be seen that there is little or no correlation between gross internal anatomy and sterility in the daughters of patroclinous males. The lone grandson of black-producing patroclinous males was found to be normal in general internal make-up also.

This seems to indicate that the difficulty must be sought for within the germ cells themselves. Black-bearing male tissue makes poor male gonads. They produce a few viable spermatozoa and these can accomplish fertilization and in some cases produce females of normal appearance, although a large percentage are defective in external anatomy. These females, for the most part sterile, contain nuclei resulting from the fusion of normal female pronuclei with abnormal male pronuclei from patroclinous males, and have, therefore, paternal grandfathers, which are anomalous relatives for females.

DISCUSSION

Any attempt to explain the causes of the production of anomalous males, their high percentage of sterility, and the almost complete sterility of their few daughters, must of necessity involve theorizing in this stage of the work. An explanation on the basis of genetic factors, with the exception of any differences associated with the orange locus, is impracticable, since analysis by segregating cultures has not been made. If there are postulated differences between the various stocks in speed of penetration of the spermatozoa and in tendency of gametes to fuse, a theory

can be formulated which will account for the production of patroclinous males and for the various percentages of patrocliny and of females. This, although it fits the facts, is not proved, and therefore is not considered of sufficient importance to be presented in detail. No other possible explanation has been found.

No proof as to the cause of sterility in the patroclinous males or in their daughters has appeared. Several theories have been considered. The male nucleus, cleaving independently in the egg, may be injured physiologically. There is also the possibility that something is lost in spermatogenesis, a chromosome or other structure which is not lost in oögenesis, so that male germ cells cannot develop into normal male gonads. An injured or incomplete spermatozoön from a patroclinous male might be able to fuse with an egg and to enter into the formation of female offspring because of the dominant characters in the egg. These females would tend to be abnormal, due to defects in the paternal half of their chromosome complex. Daughters of anomalous males correspondingly show a high percentage of freaks. Failure of the injured chromosomes from the male to function normally in synapsis would account for the sterility of the daughters of patroclinous males.

The need for further critical evidence is obvious and the present theories are advanced only tentatively.

SUMMARY

1. In *Habrobracon juglandis* (Ashmead), when black-eyed males (dominant) of Lancaster or Iowa City stocks are crossed to related orange-eyed females (recessive), some black-eyed males are produced in addition to the orange-eyed males expected by sex-linkoid inheritance.

2. When black-eyed males of Iowa City stocks are crossed with orange-eyed unrelated females of Lancaster stocks, no black-eyed patroclinous males are produced.

3. Crosses involving Iowa City stocks produce higher percentages of females and lower percentages of patrocliny than those involving Lancaster stocks alone.

4. The black-eyed patroclinous males show a higher percentage of morphological abnormalities than do males and females normally produced.

5. The majority of patroclinous males tested have been sterile; some have bred as blacks and been partially fertile; while a few mosaics have produced orange-eyed daughters and have been fully fertile.

6. The orange-eyed daughters of patroclinous males are normal in morphology and fertility. The black-eyed daughters of patroclinous males

are few in number and show a large percentage of abnormality and almost complete sterility.

7. The only adult offspring of black-eyed daughters of patroclinous males is a black-eyed male, normal in appearance and completely sterile.

8. The sterility of patroclinous males and of their black-eyed daughters does not seem to be correlated with any gross internal defect.

9. Some theories are suggested as possible explanations of the production of patroclinous males, their sterility and that of their daughters.

LITERATURE CITED

WHITING, P. W., 1918 Sex-determination and biology of a parasitic wasp, *Hadrobracon brevicornis* (Wesmael). Biol. Bull. **34**: 250-256.

1921 a Studies on the parasitic wasp, *Hadrobracon brevicornis* (Wesmael). Biol. Bull. **41**: 42-54.

1921 b Rearing meal moths and parasitic wasps for experimental purposes. Jour. Heredity **12**: 255-261.

1922 Genetic mosaics and ontogenetic abnormalities in the parasitic wasp, Hadrobracon. Proc. Amer. Soc. Zoöl., Abstract 10. Anat. Rec. **23**: 94.

1923 The analysis of genetic differences through haploid parthenogenesis. Scientific Papers of the Second International Congress of Eugenics, Sept. 1921. Vol. 1, Eugenics, Genetics and the Family, pp. 102-105.

1924 A study in hereditary and environmental factors determining a variable character. Univ. Iowa Studies. Vol. 3, No. 1. 80 pp.

HOMOLOGOUS AND SIMILAR CHROMOSOMES IN DIPLOID AND TRIPLOID HYACINTHS

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INTRODUCTION

The results of researches into the genetic consequences of the triploid or tetraploid state of one or more of the sets of homologous chromosomes, together with the genetic consequences of segmental interchange between paired homologous chromosomes, have shown that, with certain exceptions, a chromosome can be regarded, in its essentials, as a group of genes. When we find that there are two chromosomes of each size and shape, and that at the metaphase of the first maturation division the chromosomes are connected in pairs, forming independent bivalents, the two members of each pair passing to opposite poles, without interference with the members of any other pair; then we may infer that, apart from species hybrids and similar exceptions, the ordinary diploid segregation of genes is taking place. If the plant has in its chromosome group four chromosomes of each size and shape, and at the first maturation metaphase each of these sets of four chromosomes forms a quadrivalent, we may conclude that tetraploid segregation of genes will be found. On the other hand, if there are four chromosomes of each shape and size, which, however, form two bivalents that do not attract each other; then the plant may not be tetraploid, nor show tetraploid inheritance, but may have descended, either through

tetraploidy or by some as yet unknown process, from an ancestor with the same chromosome sizes, but with only half the number. It would then probably show duplicate genes. In this case it can be called a double diploid. It is, however, possible that such a chromosome grouping might have originated in some other way, without the presence of duplicate genes.

Several species of flowering plants, both dicotyledons and monocotyledons, have been examined by the writer with regard to this point. *Dahlia imperialis*, a wild species sometimes cultivated, was found to possess 16 pairs of chromosomes; while the ordinary garden Dahlia has 32 pairs (as reported by ISHIKAWA 1911). But the chromosomes of the latter could not be satisfactorily classified as to their sizes, and quadrivalents were not apparent. *Tradescantia virginiana* showed at the first maturation metaphase, in addition to many bivalents, occasional rings and strings of four chromosomes each, while a related genus is known to have half the number of chromosomes, but the sizes were not sufficiently distinct to use as a means of distinguishing homologous or similar chromosomes. In the oriental hyacinth of cultivation, however, as has long been known, there are four small chromosomes, four medium, and eight large. The problem was, therefore, to determine whether the four chromosomes of each size class were identical in length, breadth and position of constriction; and whether, if apparently identical, and associating in pairs at the first metaphase, as found by Miss HYDE (1909), there was any obvious mutual attraction between the two pairs themselves. Also, whether the eight large chromosomes were divisible into two classes of four each, as Miss HYDE'S observations seemed to indicate. Since triploid hyacinths were also known, the matter gained in interest, for the behavior of the triploid Daturas and Cannas at the first maturation division, only enlightened us as to the behavior of chromosomes united end to end in the bivalents or trivalents; whereas the constituent chromosomes of the large bivalents of the hyacinth were united at other points, and often had free ends. If trivalents occurred, those formed by the long chromosomes should be instructive with regard to the mutual connections. In addition, it was necessary to ascertain whether the size relations of short, medium and long chromosomes found in the haploid group in the pollen grain, where measurement is comparatively easy, agreed with those determined by DE MOL (1921) from the diploid group of chromosomes in the root tip. When these points were cleared up, the work might form a contribution to the evidence as to whether such a quadruple chromosome grouping had arisen through ancestral tetraploidy, through species crosses, through inherited fractures

of chromosomes at the constrictions, through permanent combinations of chromosomes, or by some as yet unsuspected process.

HISTORICAL RÉSUMÉ

OSAWA (1920) figured and briefly alluded to the attachment of some of the univalent chromosomes to bivalents in the first metaphase of a triploid Morus. In 1921, the writer described and figured pollen mother cells of a triploid Canna ("Gladiator"), in which all the 27 chromosomes were normally in trivalents at the first metaphase (BELLING 1921). Another triploid Canna ("Firebird") has since been found in the same condition. In some specially clear figures observed in late autumn, the three constituent chromosomes of each trivalent were connected by fine threads during the early anaphase; so that it could be seen that two went to one pole and one to the other, and that this was a random process, with regard to the numbers passing each way. In the next year it was shown (BELLING and BLAKESLEE 1922) that the presence of trivalents in diakinesis and first metaphase was normal in triploid Daturas. It was also demonstrated that the assortment of the 12 extra chromosomes into two groups accorded with the laws of chance, with a slight excess, however, of the more unequal divisions. *Hemerocallis fulva* (growing by the roadside at Cold Spring Harbor) appears to be a somewhat irregular triploid, in which, however, the 11 trivalents can occasionally be made out. No one apparently has observed trivalents in triploid Oenotheras. The triploid Solanums of WINKLER (1916), and the triploid mosses obtained by SCHWEITZER (1923), seem not to have been examined with regard to this point.

In 1921 DE MOL described four triploid hyacinths, but confined his observations almost wholly to the chromosomes of the root tips. The writer then began a study of the chromosomes in the pollen grains, and the bivalents and trivalents in the pollen mother cells of hyacinths, making use of the iron-acetocarmine method (BELLING 1921 b, 1923). One advantage of this method is the rapidity with which fixation occurs, though hardening of the chromosomes and cytoplasm takes some days. A criterion of good fixation for plant chromosomes is perhaps of use. The writer is inclined to consider that if the metaphase of the second division in the pollen mother cell, or the metaphase of the first division in the pollen grain, shows the longitudinal splits and the constrictions distinctly, in all the chromosomes whose positions admit of these being seen, then the fixation can not be far from complete. Applying the usual fixatives after removing the pollen mother cells from the anther, gives excellent results as to fixation, as was shown by TAYLOR (1922).

In Datura the two chromosomes of each bivalent are joined at the ends, and the same is true of the trivalents (BELLING and BLAKESLEE 1922). No clear case of any other junction has yet been seen at diakinesis or first metaphase. In Canna the bivalents and trivalents resemble those of Datura in this respect. In Hyacinthus, however, the large chromosomes in the bivalents and trivalents are connected at other points; and the bivalents often resemble the single or double rings described in certain animal maturation divisions.

That there were eight bivalents in *H. orientalis*, at the first division of the pollen mother cell, was observed 15 years ago by Miss HYDE (1909). The same observer noticed that the pollen mother cells in the bulbs underwent the maturation divisions at the end of October and during the early days of November. Three years after, root tips of the variety *albulus* were found to have 8 long V chromosomes, 4 medium, and 4 short chromosomes (MÜLLER 1912). More recently, fracture (or possibly constriction) of a chromosome was seen occasionally in the somatic divisions of the same variety (CARRUTHERS 1921). The examination of the root tips of a number of clonal varieties of *H. orientalis* (DE MOL 1921) showed 19 diploids, 4 triploids, 5 plants with fewer chromosomes, and 5 plants with more chromosomes than triploids. *Hyacinthus romanus* Desf. had only 4 pairs of chromosomes in the root tips (DE MOL 1921). Three years later it was demonstrated that the eight additional long, medium and short chromosomes were distributed at random in the pollen grains of a triploid hyacinth (BELLING 1924).

For three autumns the writer has observed 3 clonal varieties of Dutch hyacinth: (1) A diploid clone with pale bulbs and sulfur-yellow flowers, which can be easily identified. It is listed as "Yellow Hammer." (2) A triploid clone, catalogued as "Lady Derby," with purple outer bulb scales; the flowers suffused with pink, and turning red when they fade. (3) A triploid clone, listed as "King of the Blues," flowering later; with dark purple bulb scales and deep violet-blue flowers. There has appeared no cause to consider any of these three strains as different from the strains of the same names and characters examined by DE MOL (1921).

THE DIPLOID HYACINTH

Chromosomes

This plant showed 4 long, 2 medium, and 2 short chromosomes in the pollen grain (figure 1), and also at each pole at the anaphase of the second division of the pollen mother cell (figure 2). DE MOL (1921) found 8 long,

4 medium, and 4 short chromosomes in the cells of the root tips. At the metaphase of the first division in the pollen grains, only those chromosomes which happen to be horizontal can be accurately measured. This was naturally more rarely the case with the long V's than with the shorter chromosomes. In this haploid group of 8 chromosomes, two pairs of different chromosomes can be readily made out (figures 1 and 2), and the presence of four different pairs seems indicated. Of the 4 large V-shaped chromosomes, each with a clear constriction at the center of the V, 2 were sometimes seen with a second constriction (visible in three of the chromo-

FIG. 1

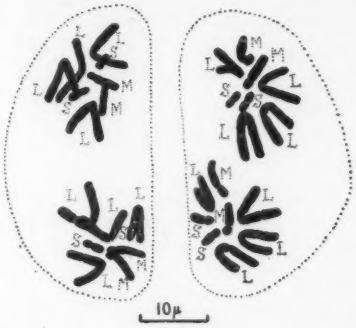

FIG 2.

FIGURE 1.—Haploid group of chromosomes in the metaphase, in the pollen grain of the diploid hyacinth, "Yellow Hammer." The constriction where the spindle fiber is attached is evident in all the chromosomes. All but one of the long chromosomes are more or less foreshortened. (Camera drawing, made with Zeiss water-immersion apochromatic objective 70; as were all the subsequent figures.)

FIGURE 2.—Four haploid groups at the late anaphase of the second division in the pollen mother cell of the diploid hyacinth. In three of the groups two of the long chromosomes show a second constriction.

some groups in figure 2); but it could not be determined whether this second constriction was invariably present in these two. The 2 medium J chromosomes had a clear constriction at the angle of the J, one-quarter of its length from one end. No difference in length was perceptible between the two similar J's, nor was any difference of shape noticed. These medium J's were about half the length of the long V's. The members of the pair of short similar chromosomes were not distinguishable from one another in length, nor were differences of shape seen. Each had a clear constriction one-third of its length from one end, and was about half as long as a medium chromosome.

To test whether there might be any small difference in length between the similar chromosomes of the haploid pairs in the pollen grain, about 20 metaphase chromosomes of each kind were drawn with the camera at a magnification of 710, and measured with dividers under a binocular magnifying 3.5. The chromosomes were selected from eight pollen grains of a triploid, and were chosen for measurement because of their horizontality. Twenty of the short chromosomes varied from 5.0 to 7.0, and averaged 5.9. They seemed to belong to one size group. Twenty-one of the medium chromosomes varied from 9.5 to 12.5, and averaged 10.5. All were in one class. But 17 of the long chromosomes varied from 18.0 to 30.5, averaging 23.1. The variation in length of the large V's was conspicuous, though care was taken only to measure those that appeared horizontal. In another pollen grain where the two groups of chromosomes at the anaphase were not far from horizontality, sufficient pressure was applied to squeeze the groups out flat, and the chromosomes were then drawn at a magnification of over a thousand, and measured under the binocular as before. The unit of measurement was twice as large as that used above. Each chromosome was matched with its fellow from which it had split, and the differences were slight, affording thus a proof of horizontality. The four small chromosomes averaged 7.2 (ranging from 6.5 to 8.2), the segments being 4.9 and 2.4. The 6 medium chromosomes averaged 13.3 (ranging from 13.0 to 13.5), the segments being 10.0 and 3.3. The 8 long chromosomes averaged 26.6 (ranging from 24.2 to 32.2), the segments being indistinguishable in length. (All these pollen grains were from the clone, "Lady Derby.") Thus in the measurements at the metaphase, the lengths of small, medium and large were as 1.0 : 1.8 : 3.9; while in the measurements at the anaphase the ratio was 1.0 : 1.8 : 3.7. These are not far from 1 : 2 : 4. (DE MOL's measurements of root-tip chromosomes gave different figures, the long chromosomes appearing relatively much shorter.) On the whole then we may consider that each of the two pairs found in the haploid group consists of two members indistinguishable in shape and size. This is also indicated, but not yet proved, for the two pairs which probably make up the four long chromosomes of the haploid. Hence, the size formula for the diploid group may be put as 8L+4M+4S, or possibly 4L+4L′+4M+4S.

Bivalents

At the first division in the pollen mother cells, as has already been described (HYDE 1909), there are found 8 bivalents: 4 large, 2 medium and 2 small (figures 3 and 4). These bivalents were in the metaphase,

good examples of the late prophase not having been found in the buds studied. The bivalents were especially examined in 51 pollen mother cells selected as showing the 8 bivalents without overlap. Only those bivalents were considered which presented clear configurations with little or no foreshortening. The long bivalents included 14 cases of the loop with two free ends, 10 X's, 5 cases of the loop with four free ends, and 2 figures of eight. The medium bivalents comprised 7 truncated A's, 5 X's or crosses, and 2 loops with free ends. The 16 short bivalents were crosses, mostly with two shorter transverse arms (figures 3 and 4). The metaphase chromo-

FIG. 3

FIG. 4

FIGURE 3—First metaphase in the pollen mother cell of the diploid hyacinth, showing the 8 bivalents. In three of the large bivalents the constituent chromosomes are connected at two points, and in the other large bivalent, at three points. The constituents of the medium and small bivalents seem connected at one point only.

FIGURE 4—First metaphase in the pollen mother cell of the diploid hyacinth, the cytoplasm with chromosomes having been squeezed from the cell. The chromosomes of two of the large bivalents are connected in two places, while the other two show only one connection.

somes were closely corrugated. At the anaphase (figure 5), each large bivalent separated, as is usual, into two split double chromosomes; the two V's of each double V being connected, after the well-known fashion, at the constriction, where the spindle fiber was attached. Each medium bivalent also separated at the anaphase in the ordinary way into two double J's, the halves being connected at their points of constriction; and the same happened to the small bivalents. These anaphase chromosomes still showed the corrugations, but became smoother before the second division in the pollen mother cell. The methods of connection, of separation, and of splitting of the two chromosomes of each bivalent have not yet been completely followed.

Similar bivalents

Fifty-one pollen mother cells in the first metaphase, selected as showing the 8 bivalents without overlap, were examined, as already mentioned. The number of times the two small and the two medium bivalents, respectively, were near enough together to be regarded as constituting a pair, was noted, as well as the number of cases in which other chromosomes were in equal proximity to any one of these. Considering first the small bivalents: there was, for the left-hand one of the two, a total of 114 potential partners, including the other small bivalent when necessary. For the right-hand small bivalents, there were 110 possible partners. The average then is 112 apparent pairings for either one of the two small

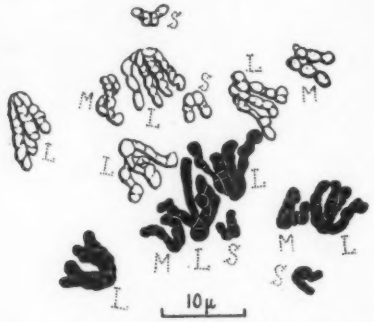

FIGURE 5.—Anaphase of first division in the pollen mother cell of the diploid hyacinth. The constituent chromosomes of the bivalents are seen to have split, forming double V's and double J's.

bivalents. On mere chance proximities, the other small bivalent should only be a partner in one-seventh of these potential pairs, because it is one out of 7 other bivalents. Hence there should have been found, on the average, 16 pairs of small bivalents. But actually there were 37 cells in which the two small bivalents were close enough together to be reckoned as a pair. The difference is 21.0 ± 2.5, which is well marked. For the medium bivalents the average number of such potential pairings was 141, giving 20.1 for the calculated number of chance proximities of two medium bivalents. Actually, 26 such pairs were found. The difference is 5.9 ± 2.8, which is not significant, taken alone.

Further, the first 16 metaphase plates were drawn with the camera, at a magnification of 520, and the distances were measured under a binocular: (1) between the two small bivalents; (2) between the two medium bivalents; and (3) between each of the two small and each of the two

medium bivalents. The average distance between two small bivalents was 4.0; that between two medium bivalents was 8.3; while the average distance between a small and a medium bivalent was also 8.3. This mutual proximity of the small bivalents might have been due, wholly or in part, to the well-known fact that the smallest chromosomes are usually nearest the center of the equatorial plate, and the largest on the periphery. To ascertain this, the distances of the bivalents from the center of the cell, as seen in sectional view, were measured for the 16 pollen mother cells in question. The average distance of the 32 small bivalents from the center was 4.2, while the average distance of the

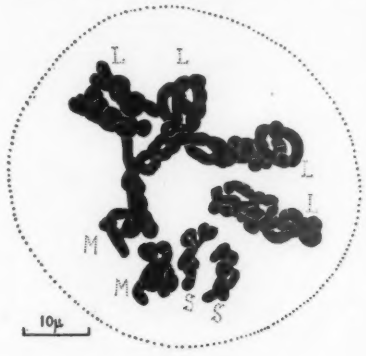

FIG. 6

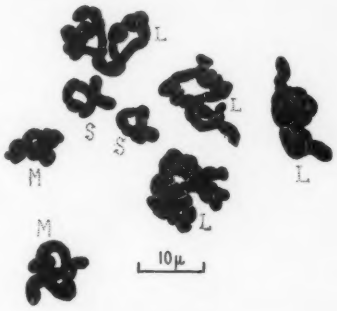

FIG. 7

FIGURE 6.—Late prophase in the pollen mother cell of the triploid hyacinth, "King of the Blues." Three of the large trivalents and one of the medium trivalents overlap somewhat.

FIGURE 7.—Late prophase in the pollen mother cell of the triploid hyacinth, cytoplasm and chromosomes having been squeezed from the cell. The three chromosomes can be traced in most of the trivalents.

medium bivalents from the center was 8.5. Hence the small bivalents were on the average only half as far from the center of the cell as were the medium bivalents. If we imagine two circles, one with a radius twice that of the other, it is obvious that two bivalents spaced at random on the outer circle should average twice as far from one to the other as would two bivalents spaced by chance on the inner circle. The distance between two medium bivalents has already been found to average about twice the distance between two small bivalents, and the mean distances between small and medium bivalents are also in agreement. Hence, the attraction between similar small bivalents, if it exists, must be slight as compared with the drift towards the center.

THE TRIPLOID HYACINTHS

The descriptions and figures refer to the clone, "King of the Blues," which presented more examples of favorable stages in the late prophase. Observations with regard to other stages made on the clone, "Lady Derby," gave similar results. These two hyacinths showed 6 short, 6 medium and 12 long chromosomes in the root tips (DE MOL 1921). One count made on each at the anaphase of the second division in the pollen mother cell seemed to confirm these numbers.

Trivalents

In the late prophase of the first maturation division there were 8 trivalents: 4 large, 2 medium, and 2 small (figures 6 and 7). No sexivalents were seen. Each long chromosome was usually connected with one or both

FIGURE 8.—a, two large trivalents from the same cell, selected to show the connections; b, two large trivalents from another cell.

of its two partners, in the comparatively few examples which showed this clearly, near one or both ends, and sometimes at intervening points. The medium chromosomes were mutually connected at fewer points, and the short chromosomes showed only 1 or 2 such junctions each. The chromosomes, especially the long chromosomes, were already more or less tortuous and somewhat corrugated. In figure 8, a and b, there are shown four trivalents of long chromosomes, from two pollen mother cells in the late prophase. In the two trivalents shown in 8a, two of the chromosomes are connected near both ends, and the third chromosome is connected with one of these junctions. The chromosomes cross and apparently fuse at several other points. These points seem to be equally distant from the connected ends of the chromosomes in question. In 8b, the chromosomes of the trivalent on the left are connected nearly end to end. In the trivalent on the right, two of the chromosomes are connected near one end

of each, and the other chromosome is free except for one obvious lateral junction. In figure 8, one of the apparently original chromosomes of each trivalent has, for the sake of clearness, been made solid black. Nothing, however, is meant to be implied by this as to what chromosomes or parts of original chromosomes separate at the anaphase.

At the metaphase, the chromosomes of the trivalents are closely corrugated, so that the points of junction can not be clearly seen. At the anaphase of the first division, the double V's, and the medium and small double J's are still corrugated, as in the diploid hyacinth. The examination which has been made of the distribution of chromosomes in the pollen grains of the clone "Lady Derby," showed that the 8 extra chromosomes of the triploid were distributed at random to the pollen grains; and that all, or nearly all, of the pollen grains with extra chromosomes went through the first nuclear division, at least as far as the metaphase (BELLING 1924).

DISCUSSION

That *H. orientalis* has four equal or nearly equal long chromosomes (probably forming two pairs), one pair of similar medium, about half as long, and one pair of similar short chromosomes, about half as long as the medium, in the haploid group, might perhaps be taken as indicating near or remote descent from a species with half the number of chromosomes (namely, two long, one medium, and one short) in the haploid group. But the allied species, *Galtonia* (*Hyacinthus*) *candicans*, also has four equal or nearly equal long chromosomes, one pair of similar medium, about half as long, and one pair of similar short chromosomes, about half as long as the medium, in the haploid group, according to the figures of NEWTON (1924). These similar chromosomes do not show mutual attraction in the somatic anaphases or telophases of Galtonia, though the homologous chromosomes do. Unlike *H. orientalis*, however, the constrictions and spindle-fiber attachments in Galtonia are close to the ends of the chromosomes (NEWTON 1924). It is hence possible that the origin of the pairs of similar chromosomes in *Hyacinthus orientalis* may date back to before the separation of the closely allied genera, Hyacinthus and Galtonia.

With regard to the variable connections observed between the constituent chromosomes of the bivalents and trivalents, it has been assumed: (1) that they are accidental twistings of chromosomes, or openings out between chromatids; or (2) that they mark points where segmental interchange has occurred or might have occurred. There does not seem to be sufficient evidence as to which of these two is the preferable working hypothesis.

SUMMARY

1. The haploid group of the diploid hyacinth probably consists of four pairs (and certainly contains two pairs) of similar chromosomes, the members of each pair being indistinguishable in size, shape and position of constrictions. The ratio of the lengths of short, medium and long chromosomes in the pollen grain averaged in metaphase and anaphase, 1 : 1.8 : 3.8, which is not far from 1 : 2 : 4. The distances of the constrictions from the ends were one-third, one-fourth and one-half of the lengths of the chromosomes, respectively.

2. There was no perceptible attraction between the members of the pairs of similar bivalents at the first maturation division. The well-marked mutual proximity of the two small bivalents was due, mainly or wholly, to their drift to the center of the chromosome plate.

3. A sample of the long bivalents showed, at the metaphase, 19 rings or loops with two or four free ends, 10 X's, and 2 figures of eight. The medium bivalents had 12 truncated A's or crosses, and 2 rings; while the small bivalents gave 16 crosses only.

4. In the triploid hyacinths there were 8 trivalents: 2 pairs of large, 1 pair of medium, and 1 pair of small. At the late prophase, in the few cases which could be followed, two of the chromosomes of a large trivalent were sometimes seen to be connected near both ends, the third being joined near its end to one of these connections. Or, all three were joined end to end; or one might be connected laterally. Intermediate connections probably occurred also.

LITERATURE CITED

BELLING, JOHN, 1921 a The behavior of homologous chromosomes in a triploid Canna. Proc. Nation. Acad. Sci. **7**: 197-201.

1921 b On counting chromosomes in pollen mother cells. Amer. Nat. **55**: 573-574.

1923 Microscopical methods used in examining chromosomes in iron-acetocarmine. Amer. Nat. **57**: 92-96.

1924 The distribution of chromosomes in the pollen-grains of a triploid hyacinth. Amer. Nat. (In press.)

BELLING, JOHN, and BLAKESLEE, A. F., 1922 The assortment of chromosomes in triploid Daturas. Amer. Nat. **56**: 339-346.

CARRUTHERS, D., 1921 The somatic mitoses in *Hyacinthus orientalis* var. *albulus*. Arch. f. Zellforsch. **15**: 370-376.

HYDE, EDITH, 1909 The reduction division in the anthers of *Hyacinthus orientalis*. Ohio Nat. **9**: 539-544.

ISHIKAWA, 1911 Eytologisch Studien über Dahlien. Bot. Mag. Tokyo. **25**; 1-8.

DE MOL, W. E., 1921 De l'existence de variétés hétéroploïdes de l'*Hyacinthus orientalis* L. dans les cultures hollandaises. Diss. Zürich. Arch. Nëèrl. Sci. Exacts et Natur. **4**: 18-143. *13 pls.*

MÜLLER, H. A. C., 1912 Kernstudien an Pflanzen, I und II. Arch. f. Zellforsch. **8**: 1-51.

NEWTON, W. C. F., 1924 Pairing and segmentation in Galtonia. Annals of Bot. **38**: 197-206.

OSAWA, I., 1920 Cytological and experimental studies in Morus, with special reference to triploid mutants. Bull. Imp. Sericult. Sta. Japan **1**: 317-369.

SCHWEITZER, J., 1923 Polyploidie und Geschlechtsverteilung bei *Splachnum sphaericum*. Flora **16**: 1-72.

TAYLOR, W. P., 1922 Organization of heterotypic chromosomes. Science N. S. **56**: 635.

WINKLER, HANS, 1916 Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeitschr. f. Bot. **8**: 417-531.

THE INHERITANCE OF A PAIR OF SPOROPHYTIC CHARACTERS IN SPHAEROCARPOS

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INTRODUCTION

It is well known that certain species of Sphaerocarpos are characterized by the persistent union of the four spores formed by the division of a spore mother cell, each tetrad being surrounded by a resistant wall. According to Miss HAYNES (1910), however, the spores of *Sphaerocarpos Donnellii* Aust. separate at maturity; HAYNES and HOWE (1923) say that the "spores in *S. Donnellii* become free at full maturity"; and AUSTIN (1877), in the original description of the species, seems to refer to the same characteristic: "Coccus deeply lobed, . . . sometimes quite fragile."

In my cultures of *S. Donnellii*, on the contrary, with exceptions noted later, the spores regularly remain united in tetrads. The wall surrounding each tetrad is comparatively thick, black at maturity, with a characteristic sculpturing distinct from that of the walls of the enclosed spores. The tetrad wall persists both at and after maturity; in the latter case, it is immaterial whether the spore-containing capsule is dried or is left in the culture until the capsule wall, the enclosing involucre, and the contiguous portions of the maternal gametophyte decay and the spore tetrads become scattered upon the soil. The spores are similarly united from the time of their formation until that of maturity; but until a comparatively late period the tetrad wall is colorless and transparent, its sculpturing apparently not having yet developed, and the outlines of the included spores and the structure of their walls are then clearly visible.

Whether this persistent union of the spores, differing from the specific character as described from out-of-door material, is consequent upon greenhouse conditions or is characteristic of particular, perhaps geographically localized, races, is still uncertain. Each of these possible explanations finds some support in the observations described below. My cultures originated in material from Sanford and Miami, Florida; the type locality for the species is Jacksonville, Florida.

The work here discussed has been aided by grants from the research fund of the UNIVERSITY OF WISCONSIN; and I have had the assistance, at various periods, of Messrs. H. W. RICKETT, A. M. SHOWALTER and J. A. LOUNSBURY. A portion of the results have already been briefly reported (ALLEN 1923).

THE SEPARATE-SPORE CHARACTER

In 1919, it was observed that the spores borne by the sporophytes resulting from a mating in which clone R27E (which began with an isolated tufted female plant from Sanford, Florida) was the female parent, were not united in the manner that had thus far appeared characteristic of the species. This fact was noted in an earlier paper (ALLEN 1919, p. 294, footnote), and the results, with respect to other characters, of this and other matings of clone R27E have since been reported in detail (ALLEN 1924, 1925). In all, five matings have been made of this clone, all with like results. The males concerned represented three distinct clones. Two of these three male clones (R19δ1aB and 19.27) have also been mated with numerous other female clones, such matings invariably resulting in sporophytes whose spores were united in tetrads. It is thus evident that the separateness of the spores borne by the sporophytic offspring of clone R27E is not a result solely of exceptional external influences; it represents, on the contrary, a tendency inherent in, or inherited through, this maternal clone. The union or non-union of the spores will for the present be treated as a sporophytic character, which it apparently is; although it is true that this character may conceivably be rather an expression of the genotypic constitution of the maternal gametophyte, influencing in some way the development of spores within the attached and dependent sporophyte.

For simplicity of statement, spores firmly enclosed within a tetrad wall will be spoken of as "united"; those not so enclosed, as "separate." In the latter case, when they are released by a breaking of the capsule wall, some, though never a considerable proportion, of the spores often retain the tetrahedral arrangement; but a touch separates them. Sometimes, when such adherent spores are separated, there appear what may be fragmentary

remnants of the original mother-cell wall; but nothing showing the thickness, color, or structure of the wall that surrounds each tetrad of "united" spores. If a capsule of a separate-spore strain is immature and the spores are still green or greenish, they sometimes manifest a greater tendency to adhere in tetrads than at a later stage; but in such a case, also, they are easily separable. On the other hand, in those instances in which the spores are described as "united," the tetrad wall can be ruptured only by a considerable pressure, which is about equally likely to break the walls of the individual spores. Thus, save for the very few exceptional cases mentioned below, the sporophytes resulting from all the matings that have been carried out fall into two sharply distinct categories: they have produced either "united" or "separate" spores. The difference seems to result from the development in the one case, and the non-development in the other, after the division of the protoplast of the spore mother cell, of a tetrad wall in addition to, or replacing, the mother-cell wall.

In no instance has any marked difference with respect to union or separateness of spores been observed between the various sporophytes resulting from a single mating. As a general rule, several to many sporophytes from each mating have been examined; a few matings produced only one or two sporophytes each.

Sporophytes have been obtained and examined that resulted from 113 matings in which neither parent was clone R27E or a descendant of that clone. Typical (♀ and ♂), tufted (♀ and ♂), and polycladous (♂) plants were involved. Of the 113 matings, 3 had occurred in mixed cultures before they were received; 12 were brought about within mixed cultures or between portions of two such cultures after their receipt from Sanford or Miami; 5 were between female clones (each developed from an isolated plant, or from a spore from an earlier mating) and male plants from the original cultures; and the remaining 93 were between clones of various origin. The sporophytes borne by 110 of the 113 matings in question bore uniformly united spores.

Of the three matings whose results were more or less divergent, two were of a single female clone (21.215) with different males. The sporophytes resulting from these two matings bore spores usually separate; but the occasional tetrads could sometimes be seen to be surrounded by a thin wall, evidently fragile but with the markings characteristic of a tetrad wall and distinct from those of the walls of the enclosed spores. In respect of the enclosing wall, then, these tetrads of adherent spores seemed to differ from those sometimes obtained from sporophytes descended from clone R27E. Matings of clone 21.215 with two other male clones resulted

uniformly in sporophytes bearing united spores. The third mating giving divergent results involved female clone 20.339; most of the spores were united, but a few were separate. In a mating of the same female with another male clone, only united spores were observed to be produced. Apparently, this and the two previous cases are to be explained by an incomplete development of the tetrad wall, perhaps in consequence of external conditions. They may suggest, too, that conditions in the natural habitat of the species are such as to check or inhibit the development of the tetrad wall, thus accounting for the observations already cited from the taxonomic literature. It is clear, at any rate, that the inherent tendency to the production of uniformly separate spores distinguishes clone R27E from all others thus far studied in culture.

THE F_2 GENERATION

If clone R27E and the males with which it was mated are considered the starting-point (the p_1 generation, following the symbolism used in previous papers), the sporophytes resulting from those matings belonged to the F_1 generation. These sporophytes, as has been seen, bore separate spores. From the germination of their spores arose the gametophytes of the f_1 generation. The mating of f_1 gametophytes, in turn, gave rise to F_2 sporophytes.

One hundred sixty-six matings that produced sporophytes have been made, in which one or both partners were f_1 progeny of R27E, and therefore of F_1 sporophytes bearing separate spores. Except in a few cases in which the material was too scanty, each f_1 female clone descended from R27E was mated with males of the same and of different origin; and each f_1 male clone so descended, with a few exceptions, was mated with females of the same and of different origin. Among the females used in these matings were typical and tufted clones; and among the males were typical, tufted and polycladous clones.

Of these 166 matings, 88 were between females and males, both progeny of R27E; these involved 47 female clones, of which 44 were progeny of mating 8 previously reported (ALLEN 1924), 1 of mating 10 (ALLEN 1924), and 2 of mating 15 (ALLEN 1925); and 27 male clones, of which 18 were progeny of mating 8, 2 of mating 10, and 7 of mating 15. In every case, the resultant F_2 sporophytes bore separate spores.

Sixty matings were between f_1 female progeny of R27E and males of other origins; 45 female clones were concerned, of which 42 were progeny of mating 8, one had resulted from mating 10, and 2 were progeny of mating 15. All the F_2 sporophytes produced by these matings likewise

bore separate spores. Thus all the f_1 female descendants of clone R27E, whether typical or tufted and however mated, gave rise, like the ancestral female clone, to sporophytes with separate spores.

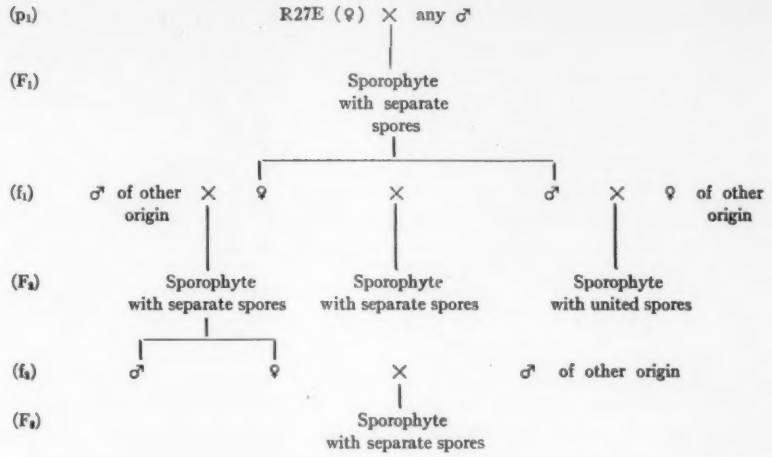
Eighteen matings were between f_1 male progeny of clone R27E and females of other origins. Seventeen male clones were involved; 15 were progeny of mating 8, 2 of mating 15. The F_2 sporophytes resulting from 16 of these matings uniformly bore united spores. In one of the two exceptional cases, three separate spores were seen, all the others observed being united; this instance may perhaps be classed with the exceptional cases noted in an earlier category, as due to a partial or entire failure of the tetrad wall to develop even when the possibility of such development is inherent. The other instance of divergent behavior can not be thus explained. The F_2 sporophytes resulting from this mating (22.86×20.88) bore uniformly separate spores, like the F_1 sporophyte from which the male clone here concerned was descended, although in the ancestry of the female clone only united spores had appeared.

THE F_3 GENERATION

Seven matings have been made between 5 f_2 female descendants of clone R27E (progeny of mating 11) and males of other origins. The maternal f_1 ancestor (20.124) of these f_2 females had produced sporophytes with separate spores in matings with two males of similar, and three males of different, origin (all included among the matings above reported). Each of the 7 matings of the f_2 females resulted in F_3 sporophytes with separate spores. Thus the separate-spore character had been transmitted from the p_1 female (R27E) through an F_1 sporophyte, an f_1 *female* gametophyte, an F_2 sporophyte, and an f_2 *female* gametophyte, to the F_3 sporophytes.

DISCUSSION

Apart from one distinctly exceptional case, the inheritance of the alternative separate-spore and united-spore characters among the descendants of clone R27E, so far as it has been observed, has followed the course shown in the following scheme:



The tendency of a sporophyte of whatever generation to produce separate or united spores is inherited through the maternal, and not (with one apparent exception) through the paternal, gametophyte. The transmission of either of these alternative tendencies, therefore, parallels the transmission of the X chromosome from female gametophyte to sporophyte, and thence through the latter's gametophytic offspring to the sporophyte of the succeeding generation. Whether a character expressed in the asexual generation of a bryophyte should be termed "sex-linked" is questionable; but the pair of alternative characters here considered evidently bear essentially the same relation to the X chromosome that the sex-linked characters of an animal bear to its X (or Z) chromosomes. Apparently, in view of recent descriptions of sex chromosomes in Lychnis, the comparison is equally close with the sex-linked character in that plant described by BAUR (1912) and more fully studied by SHULL (1914).

If the union or separation of the spores is to be considered as imposed upon the parasitic sporophyte by the genotypic constitution of the maternal gametophyte,—and thus, in effect, a gametophytic rather than a sporophytic character,—its relation in inheritance to the X chromosome is equally obvious. From this point of view, being a character of the sexual generation, it would be "sex-linked" in the strictest sense.

The close linkage of this pair of characters with sex is contrasted with the entire absence of linkage between either of the pair on the one hand and tuftedness or polyclady on the other. This result agrees with previ-

ously reported observations, which indicate that tuftedness and polyclady are inherited independently of sex, as well as independently of each other.

The occurrence of separate spores in the sporophytes resulting from one mating (22.86×20.88), whereas all other results lead to the expectation of united spores in these sporophytes, might be explained by a mutation in the female clone (22.86). However, discussion of this problem may well await further results which will eliminate all possibility of an error in the records or of one due to contamination of the cultures. The general rule obtaining in the inheritance of the characters here discussed is clearly demonstrated.

SUMMARY

1. The sporophytes resulting from the mating of one female clone (R27E) of *Sphaerocarpos Donnellii* with any male bear separate spores; the spores of sporophytes produced by other matings are regularly (under greenhouse conditions) united in tetrads, each tetrad being surrounded by a resistant wall.

2. The formation of a tetrad wall is rarely (in culture) partially or completely inhibited, probably in consequence of external conditions, in strains in which the possibility of producing such a wall is inherent.

3. The separate-spore tendency is regularly transmitted, among the descendants of clone R27E, through the female gametophytes. With one possible exception, the genotypic constitution of a male gametophyte has no influence upon the union or non-union of the spores of its sporophytic progeny.

4. The transmission of the tendency to produce separate or united spores follows the same course as does the transmission of the X chromosome. This pair of characters is thus comparable with the sex-linked characters of animals and of Lychnis.

5. The characters of the pair in question are independent in inheritance of the tufted *versus* non-tufted and of the polycladous *versus* non-polycladous pairs.

LITERATURE CITED

ALLEN, C. E., 1919 The basis of sex inheritance in Sphaerocarpos. Proc. Amer. Philos. Soc. **58**: 289-316. *28 text figs.*

1923 An apparently sex-linked sporophytic character in Sphaerocarpos. (Abstract). Anat. Rec. **26**: 388, 389.

1924 Gametophytic inheritance in Sphaerocarpos. I. Intraclonal variation, and the inheritance of the tufted character. Genetics **9**: 530-587. *60 text figs.*

1925 Gametophytic inheritance in Sphaerocarpos. II. The polycladous character. Genetics **10:** 1-16. *11 text figs.*

AUSTIN, C. F., 1877 New Hepaticae. Bull. Torrey Bot. Club **6:** 157, 158.

BAUR, E., 1912 Ein Fall von geschlechtsbegrenzter Vererbung bei *Melandrium album*. Zeitschr. indukt. Abstamm. u. Vererb. **8:** 335, 336.

HAYNES, CAROLINE C., 1910 *Sphaerocarpos hians* sp. nov., with a revision of the genus and illustrations of the species. Bull. Torrey Bot. Club **37:** 215-230. *Pls. 25-32.*

HAYNES, CAROLINE C., and HOWE, M. A., 1923 Sphaerocarpales. North American Flora **14**[1]**:** 2-6.

SHULL, G. H., 1914 Sex-limited inheritance in *Lychnis dioica* L. Zeitschr. indukt. Abstamm. u. Vererb. **12:** 265-302. *5 text figs., pls. 1, 2.*

THE CHROMOSOME NUMBER OF MAIZE[1]

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HISTORICAL

The wide-spread interest in the genetics of maize, coupled with the uncertainty as to the number of chromosomes occurring in this species prompted the investigation which is here reported.

From a review of the literature it appears that KUWADA (1911, 1915, 1919) is the only worker who has reported extensive counts of maize chromosomes. His results are summarized in table 1. Variations were reported in the number of chromosomes both within and between varieties as well as between different cells of individual plants. He concluded that sweet varieties are usually characterized by having twelve chromosomes, and starchy varieties by having ten, as the haploid number. Several exceptions were recorded, as shown in the table.

With one exception his sweet × starchy hybrids were considered as having a haploid number of ten chromosomes. The pollen mother cells of a single plant of Black Mexican had one univalent and eleven bivalent chromosomes, while cells of the root-tip had twenty-three chromosomes. He considered that this had been caused by non-disjunction. According to KUWADA'S counts the haploid number varied as much as six chromosomes within some varieties.

Since completing the investigation which follows, a brief paper has appeared by LONGLEY (1924) concerning chromosome numbers in maize and related species. Four varieties including Chinese and Tepic maize are reported as each having a haploid number of 10 chromosomes.

[1] Contribution from the Department of Agronomy, NEBRASKA AGRICULTURAL EXPERIMENT STATION as paper No. 6, Journal Series. Published with the approval of the Director.

TABLE 1

Tabulation of KUWADA'S *results showing the frequency of different haploid and diploid chromosome numbers in varieties and hybrids of maize (Zea Mays) and other plants.*[2]

VARIETY OR TYPE	CHROMOSOME NUMBERS PER CELL															Number accepted by KUWADA	
	Haploid (in pollen mother cells)								Diploid (in cells of root-tips)								
	7	8	9	10	11	12	13	14	19	20	21	22	23	24	25	Haploid	Diploid
A. Maize								Frequency									
Black Starch	1	1	2	46	...	...	...	...	...	25	...	...	...	...	...	10	20
Amber Rice Pop	...	...	...	*	1	...	...	...	...	24	...	...	...	...	...	10	20
Sugar Corn	...	...	2	4	11	69	8	6	...	24	4	13	...	2	...	12	...
Black Mexican	...	...	...	...	...	*	...	...	...	11	19	34	21	27	5	12	...
Black Mexican (1 plant)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	11†	23
Red Sugar Corn	...	...	...	...	*	*	...	...	...	...	...	...	...	...	...	10	...
Early Eight Sugar Corn	...	...	*	...	...	*	...	...	...	...	...	...	...	...	...	12	...
Proterogynous Corn	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
Chinese Corn	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
Pod Corn	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	20
Hybrids	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Sugar×Black Starch	...	...	3	44	7	...	...	...	...	18	86	1	...	...	...	10	...
Amber Rice Pop × Sugar	...	...	1	6	8	65	...	...	...	...	...	...	...	...	...	12	...
Amber Rice Pop × Black Mexican	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	10	...
Black Mexican × Black Starch	...	...	...	...	...	...	...	...	...	...	...	39	...	...	...		
Sugar × Black Mexican	...	...	...	...	...	...	...	...	3	107	43	76	3	...	...	...	...
Maize × Teosinte	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
B. Other species belonging to the tribe Maydeae																	
Teosinte (annual)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	20
Coix	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	20
C. Species of the tribe Andropogoneae																	
Sorghum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
Ischaemum anthephoroides	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	68
Saccharum officinarum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	68
Saccharum spontaneum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	68

* Frequency not stated.

† This plant was reported as having 11 bivalent chromosomes and a univalent chromosome.

[2] This table is summarized by the authors from KUWADA'S tables and text.

EXPLANATION OF PLATE 1

FIGURES 1 to 6 are photomicrographs of the pollen mother cells of maize, showing chromosomes (diakinesis stage).

FIGURE 1.—Dent maize (Pride of Saline). ×400

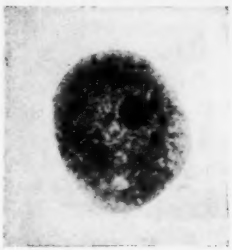
FIGURES 2 and 3.—Dent maize (Hogue Yellow Dent). ×400

FIGURE 4.—Sweet maize (Country Gentleman). ×500

FIGURE 5.—Waxy maize (Chinese). ×400

FIGURE 6.—Teosinte (annual). ×500

FIGURE 7.—Pollen mother cells of teosinte, showing abundance of material often secured in a single mount. ×200



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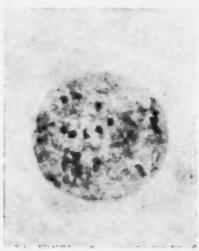

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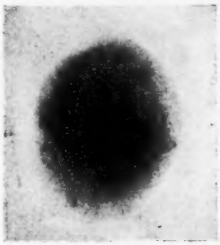

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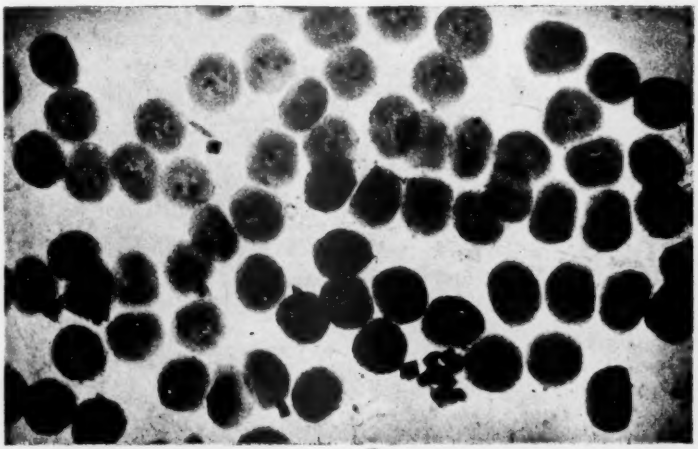

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EXPERIMENTAL METHODS

Under these circumstances it was thought desirable to count the chromosomes in a rather large number of different types of maize. The aim was to have extreme types represented, differing in plant size, earliness, leafiness, endosperm type, heterozygosity, etc. The varieties used were all grown under normal field conditions at the NEBRASKA EXPERIMENT STATION during the season of 1924. The chromosome counts were all made in pollen mother cells stained by BELLING'S (1921) iron-aceto-carmine method. Since the reduction division takes place some time before the tassel is exserted, it was difficult to tell in the field when plants were in the desired condition. Several stalks were taken to the laboratory at a time and kept in water until they could be examined.

The most advanced flowers of the central spike occur a short distance below the tip, and the least developed at its base. For some time a region showing all stages of meiosis may be found between these points. After all the flowers of the central spike are too old, good stages may still be found on the branches of the tassel but are more difficult to locate.

Young anthers not yet showing yellow coloration were placed on a slide in a drop of aceto-carmine solution and cut into two or more pieces. Sufficient iron was dissolved from the razor blades and needles used during the process. A cover glass was applied and tapped until the pollen mother cells were forced out of the anthers. If they appeared to be in the right stage the cover was taken off, the anther fragments removed, the cover replaced and sealed with a mixture of vaseline and paraffin melted with a hot knife. The slides were then left till sufficiently stained when the counts were made. Crushing the pollen mother cells as suggested by BELLING was resorted to only in cases where they showed less than the normal number of chromosomes, to see if such cases were actual variations of number or merely due to inability to see all the chromosomes.

RESULTS

All the forms studied were found to have ten as the haploid number, as is shown in table 2. This does not prove that there may not be forms of maize with different chromosome numbers, but it seems likely that the extreme variability reported by KUWADA was largely due to errors of counting. Various workers, including KUWADA, have called attention to the difficulty of making correct chromosome counts. Among the causes leading to errors, which they have mentioned, are: close contact between chromosomes making them appear as one; obscuring of some chromosomes

by others or by the nucleolus; the presence of fragments of the nucleolus or other bodies which may be mistaken for chromosomes; and in sectioned material, parts of chromosomes may be mistaken for whole chromosomes.

TABLE 2

Number of chromosomes found in the pollen mother cells of commercial varieties and pure-line (inbred) strains of maize (Zea Mays). Nebraska Experiment Station, 1924 crop.

VARIETY OR TYPE	DESCRIPTION OF VARIETY					CHROMOSOME	
	Endosperm type	Plant height inches	Leaf area per plant (sq. in.)	Days till tasseling	Days till mature	Number of counts	Chromosome number (haploid)
A. Varieties and strains of dent maize							
Commercial varieties							
Calico (North Platte)	Dent	98	980	64	97	80	10
Pride of the North	Dent	98	1092	62	103	10	10
Nevada White	Dent	87	1081	67	105	20	10
Substation White	Dent	94	979	64	106	3	10
Reid Yellow Dent	Dent	120	1426	71	112	50	10
Hogue Yellow Dent	Dent	112	1325	79	120	100	10
Ramosa	Dent	108	957	79	114	32	10
Esperanza	Dent	110	1050	80	123	50	10
Pride of Saline	Dent	122	1751	88	124	65	10
Douthit Prolific	Dent	133	2405	79	131	27	10
Mexican June	Dent	133	2493	94	143	50	10
Inbred strains							
Hogue No. 8	Dent	70	631	87	120	4	10
Hogue No. 724	Dent	90	1442	95	138	26	10
Hogue No. 726	Dent	88	631	80	123	12	10
Hogue No. 731	Dent	90	883	82	127	25	10
Hogue No. 742	Dent	75	978	85	138	8	10
Hogue No. 745	Dent	70	1171	88	132	10	10
Nebr. White Prize No. 659	Dent	70	609	77	127	25	10
Nebr. White Prize No. 676	Dent	77	1014	88	127	50	10
B. Endosperm types other than Dent							
Gehu	Flint	48	542	48	84	20	10
White Australian	Flint	83	722	59	99	50	10
King Phillip	Flint	105	1260	69	120	50	10
White Pearl	Pop	88	871	65	99	49	10
Red Flour	Soft	83	702	54	88	10	10
Country Gentleman	Sweet	90	670	56	104	91	10
Chinese maize	Waxy	85	1200	82	132	40	10
C. Plants other than maize							
Teosinte (annual)						25	10
Sorghum						15	10

By the use of BELLING's method all errors due to cutting are avoided, and the method is so rapid that it is easy to get enough material so that it is not necessary to use any but the best stages.

KUWADA accepted COLLINS's (1912) theory that maize originated as a cross between teosinte and a member of the Andropogoneae, and thought he could distinguish the larger chromosomes derived from the teosinte from the smaller chromosomes derived from the other parent species. Nothing was found which would tend to confirm this. The chromosomes of teosinte appeared very similar to those of maize, but no measurements were made to see if they were as variable in size as those of maize.

Since such great constancy was found in the number of chromosomes in widely different types of maize, it appears likely that the variability of this species has resulted from factor mutations rather than from irregularity in chromosome behavior during mitosis leading to change of number.

LITERATURE CITED

BELLING, J., 1921 On counting chromosomes in pollen mother cells. Amer. Nat. **55**: 573-574.

COLLINS, G. N., 1912 The origin of maize. Jour. Washington (D. C.) Acad. Sci. **2**: 520-530.

KUWADA, Y., 1911 Meiosis in the pollen mother cells of *Zea Mays* L. Bot. Mag. Tôkyô **25**: 163-181.

1915 Über die Chromosomenzahl von *Zea Mays* L. Bot. Mag. Tôkyô **29**: 83-89.

1919 Die Chromosomenzahl von *Zea Mays* L. Jour. Coll. Sci. (Tôkyô Imperial University) **39** (Article 10): 1-148.

LONGLEY, ALBERT E., 1924 Chromosomes in maize and maize relatives. Jour. Agric. Res. **28**: 673-681.

THE OCCURRENCE OF STARCH AND ERYTHRODEXTRIN IN MAIZE AND THEIR SEGREGATION IN THE POLLEN OF HYBRIDS[1]

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INTRODUCTION

A variety of maize (*Zea Mays*) differing from other known sorts in the texture of its endosperm was described by COLLINS (1909). The type of endosperm of this variety was designated as "waxy" in contrast with the starchy and sweet types of other maize. It has since been shown by WEATHERWAX (1922) that the carbohydrate of this waxy endosperm is erythrodextrin which can be distinguished from other carbohydrates by its red color reaction with iodine.

It has recently been reported by DEMEREC (1924) and BRINK and MACGILLIVRAY (1924) that this carbohydrate is also present in the pollen of this Chinese waxy variety. With plants homozygous for endosperm type, these authors found that iodine stains the pollen from starchy and waxy maize blue and reddish, respectively. When the pollen from plants heterozygous for these characters was stained with iodine, nearly equal numbers of blue and reddish pollen grains resulted.

They interpreted this as a segregation of the waxy and starchy characters in the pollen grains. LONGLEY (1924) has reported such segregation, also determined by the iodine test, in crosses of Chinese maize with starchy maize, and with annual and perennial teosinte, and in crosses between starchy and glutinous Coix. Similar results had previously been reported by PARNELL (1921) for glutinous and starchy varieties of rice and their hybrids.

[1] Contribution from the Department of Agronomy, NEBRASKA AGRICULTURAL EXPERIMENT STATION as paper No. 7, Journal Series. Published with the approval of the Director.

EXPERIMENTAL RESULTS

In experiments conducted at the NEBRASKA EXPERIMENT STATION during the summer of 1924 results were obtained which indicate that differences in staining of maize pollen with iodine should not be interpreted as segregation. In all maize tested, including varieties homozygous for starchy, sweet, and waxy endosperm types, as well as in F_1 hybrids of a waxy by starchy (Chinese $\times$ yellow flint) cross, all normally developed mature pollen gave the starch reaction with iodine. Likewise when mature pollen grains of either type were crushed, no difference was noted in the color reaction of their contents when stained with iodine. In all varieties, immature pollen which had not yet developed starch, stained reddish with iodine solution. This reddish color of immature pollen which is not limited to maize but is common in many other species, seems to be due largely if not entirely to staining of the walls of the pollen rather than the cell contents, and is later obscured by the dark stain of the starch. In sectioned pollen grains the wall stained red with iodine. The lignified tissue of the bundles of stems and leaves of maize also stain reddish with iodine solution.

Starch formation is not always simultaneous for all the pollen of an anther. This may result in a transition stage in which some of the pollen grains stain reddish and some blue. Such a condition could easily be interpreted as segregation into starchy and non-starchy pollen. At a later stage such pollen all gives a blue reaction. It would appear that the interpretation of variation in the color reaction of pollen to iodine, by the investigators cited, may be accounted for by the absence of a comparative study of pollen at various stages of maturity.

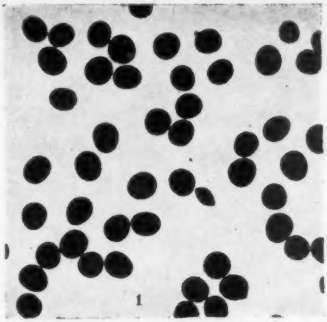
In connection with this work it was thought desirable to see if Chinese maize had erythrodextrin instead of starch in other parts of the plant, and to compare this with conditions in starchy and sweet maize. Except in the endosperm, starch has the same distribution in Chinese maize as in the starchy and sweet varieties with which it was compared. As shown by the iodine test, starch occurs in the root-tips, in young leaves and stems, persisting in the stem for a long time in the regions just above the nodes. In young ears prior to fertilization, starch was found in the shank, cob, silk, and in the kernels, where it was most abundant in the pericarp. Starch occurs throughout the very young tassels. In older tassels it occurs above the nodes of the rachis, and in the subepidermal layer of the anthers, as well as in the mature pollen.

The seed from which the pure Chinese maize was grown was obtained

Explanation of Plate 1

All figures are photomicrographs of pollen stained comparably with iodine in chloral hydrate solution.

Figure 1.—Pollen of starchy maize at shedding stage.
Figure 2.—Pollen of "waxy" Chinese maize at shedding stage.
Figure 3.—Pollen of F_1 hybrid between Chinese and flint maize at shedding stage.
Figure 4.—Pollen of F_1 hybrid of Chinese × flint maize at the transition stage when the grains show a difference in staining which might be mistaken for segregation.
Figure 5.—Pollen from hybrid at same stage as in figure 4, still in the anther.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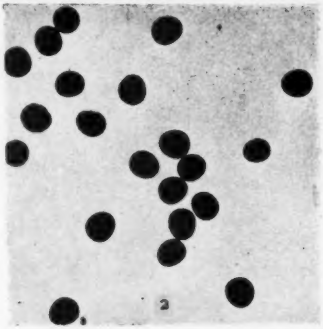
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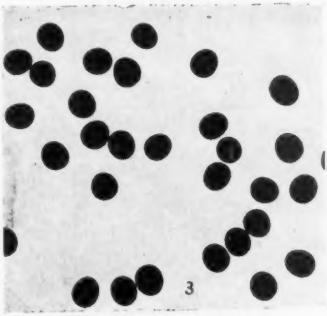
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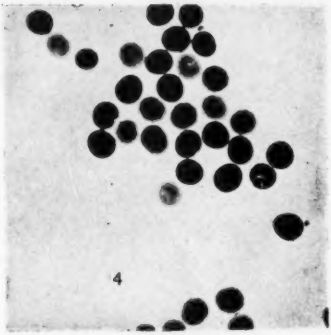
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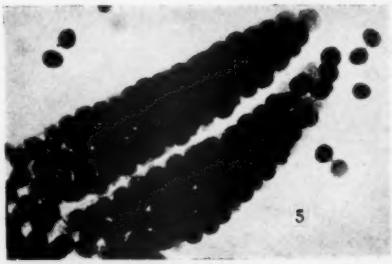
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from COLLINS last spring. That involved in the hybrids was obtained from the same source in 1922.

The investigators cited have used different forms of iodine solutions and all have obtained similar color reactions. We have used three forms of iodine solutions, namely (1) ordinary iodine in potassium iodide, (2) alcoholic iodine solution, and (3) a solution of chloral hydrate to which iodine solution had been added. Comparable results were had for all forms of solution, though the iodine solution in chloral hydrate proved advantageous especially for pollen in the anthers because of its more rapid penetration and clearing qualities.

LITERATURE CITED

BRINK,[2] R. A., and MACGILLIVRAY, J. H., 1924 Segregation for the waxy character in maize pollen and differential development of the male gametophyte. Amer. Jour. Bot. **11**: 465-469.

COLLINS, G. N., 1909 A new type of Indian corn from China. U. S. Dept. Agric., Bur. Plant Industry Bull. 161, pp. 1-30.

DEMEREC,[2] M., 1924 A case of pollen dimorphism in maize. Amer. Jour. Bot. **11**: 461-464.

LONGLEY, ALBERT E., 1924 Chromosomes in maize and maize relatives. Jour. Agric. Res. **28**: 673-681.

PARNELL, F. R., 1921 Note on the detection of segregation by examination of the pollen of rice. Jour. Genetics **11**: 209-212.

WEATHERWAX, P., 1922 A rare carbohydrate in waxy maize. Genetics **7**: 568-572.

[2] BRINK's results were briefly given in Wisconsin Agric. Exp. Sta. Bull. 362, pp. 108-109, and DEMEREC's observations were mentioned by JONES in the Bot. Gaz. **75**: 427-428.